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SELF FERTILIZATION AND POPULATION VARIABILITY IN THE HIGHER PLANTS[1]

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INTRODUCTION

In his book, "The Effects of Cross and Self Fertilization in the Plant Kingdom," Charles Darwin (1877) stated "that it is a general law of nature that flowers are adapted to be crossed, at least occasionally, by pollen from a distinct plant." Although he described in detail many instances of the operation of this so-called "law" and showed how violations of it, in the form of artificial self fertilization by man of naturally outcrossing species, led to sterility or to the production of greatly weakened offspring, he could not offer a reasonable explanation of its existence. Moreover, he was at a loss to explain why some plants, like the garden pea, are able to violate this "law" for many generations without suffering ill effects. He grouped this situation "with many facts, which are so obscure that we stand in awe before the mystery of life."

With the discovery and rediscovery of Mendel's laws of particulate inheritance, the foundation was laid for understanding the meaning of nature's apparent insistence on cross fertilization, as well as her tolerance of numerous exceptions to this law. These phenomena, however, could not be fully understood until Mendelian principles were applied to studies of populations by Fisher (1930), Wright (1931), Haldane (1932), Muller (1932) and others. In the words of Muller, these early studies of population genetics gave a definite answer to the question, "is sex necesasry." They showed that the primary function of sexual reproduction is to maintain and increase genetic diversity in populations. In a population of asexually reproducing organisms, each successive new mutation can produce only one new genotype, and consequently the increase in genetic variability must be very slow, unless the number of individuals is enormously large and reproduction

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is very rapid in terms of number of generations per unit of time. In a population of sexually reproducing organisms, on the other hand, each new mutation can, after a few generations of outcrossing, give rise to hundreds or even thousands of new genotypes, if the store of genetic variability already existing in the population is great, and if free recombination of genes is possible.

For these reasons sexual reproduction can act efficiently to increase or maintain variability only in genetically heterogeneous, cross fertilizing populations. Hence, the most logical explanation for the widespread prevalence of sexual reproduction and obligate cross fertilization in natural populations is that over long periods of time in the geological sense only those evolutionary lines have been successful which have acquired mechanisms enforcing sexuality and cross fertilization.

These conclusions, however, provide no explanation for the obvious success of many groups of plants which are predominantly self fertilized, and of which the great majority of individuals are homozygous for all or nearly all of their gene loci. They provide no clue which would help us to understand for instance, why the scarlet runner bean (*Phaseolus coccineus*) has evolved successfully through the enforcement of cross fertilization by self incompatibility, while the related garden bean (*Phaseolus vulgaris*) and lima bean (*P. lunatus*) have been equally successful as a series of self fertilizing homozygous lines. Faced with this situation and many others like it, we must recognize the fact that many plant species can be very successful in spite of persistent self fertilization with its consequent homozygosity, even though they have close relatives which require cross fertilization. One possible explanation of this apparent paradox is that the self fertilizers have been the victims of an unlucky accident. Through acquiring chance mutations, they have lost their capacity for cross fertilization. A few genotypes derived from the original heterozygous population have managed to survive and remain healthy despite the usually deleterious effects of inbreeding, but these have sacrificed their evolutionary capacity for evolutionary change, and are "dead ends" of evolution which will become extinct if in the future they become subjected to radical changes in the environment.

An alternative explanation, however, is that the evolution of self fertilization in plants is not purely accidental, but is actually favored by natural selection because self fertilizers possess some advantages over cross fertilizers under certain conditions of existence. If this is the case, then the difference between cross and self fertilization should be correlated with other differences between the plants which possess these divergent genetic systems. Several years ago (Stebbins 1950) I pointed out the existence of such correlations, and they will be treated more fully in the present discussion. They tell us that natural selection can direct not only the evolution of visible differences between populations, but also the evolution of population structure, and particularly of those mechanisms which affect the store of variability present in populations, as well as its method of release.

TYPES OF BREEDING SYSTEM IN THE HIGHER PLANTS

The flowering plants provide us with particularly good material for studying the evolution of genetic systems which affect the amount of cross fertilization in populations, since different systems often exist in related species of the same genus. An example is the grass genus Bromus, of which a few representative species are listed in table 1. Some of these, particularly the Old World species *B. erectus* and *B. inermis*, are self incompatible and well equipped for cross pollination, with large anthers which are well extruded from the florets and large, feathery stigmas. Others, particularly the perennial species of the New World *B. Orcuttianus, B. grandis, B. laevipes, B. purgans* and *B. anomalus,* have large or medium sized anthers which are usually extruded, but are self compatible, and so may often be self fertilized through passage of pollen from one floret to another of the

TABLE 1

GROWTH HABIT, ANTHER SIZE, AND POLLINATION BEHAVIOR IN SOME SPECIES OF BROMUS

Species	Growth habit	Anther Size	Pollination behavior
B. inermis	Rhizomatous perennial	Large	Self incompatible, inbreeding depression
B. erectus	Caespitose perennial	Large	Self incompatible
B. orcuttianus	Caespitose perennial	Large	Self compatible, easily outcrossed
B. laevipes	Caespitose perennial	Intermediate	Self compatible, often outcrossed
B. ciliatus	Caespitose perennial	Small	Usually self pollinated
B. mollis	Annual	Small	Usually self pollinated
B. tectorum	Annual	Small	Usually self pollinated

same plant. A third group of species represented by a few perennials (*B. ciliatus*) and by all of the annuals in the sections Bromium and Eubromus, are regularly self fertilized through their possession of small anthers which are only rarely extruded, usually depositing their pollen directly on the stigma of the same floret. Outcrossing in these species is a rare event, although it probably takes place occasionally in all of them. Finally there is a group of species in the New World sections Ceratochloa and Neobromus which, as shown by Harlan (1945a), alternately produce florets with large anthers and stigmas well suited to cross pollination, and cleistogamous florets with small anthers, in which selfing is obligatory.

Genera which, like Bromus, possess a variety of different pollination mechanisms among their various species, are numerous and widespread among flowering plants. One can mention Hordeum, Elymus, Agropyron, Festuca, Poa, and many others among the grasses; Medicago, Trifolium, and Phaseolus in the Leguminosae; Solanum, Lycopersicum, and Nicotiana

in the Solanaceae; and in other families Linum, Oenothera, Clarkia, Statice, Limonium, Gilia, Amsinckia, Madia, and Crepis. Since much is known about the genetic and evolutionary relationships of species in most of these genera, an intensive study of the pollination differences which exist between their species in relation to the other interspecific differences should shed much light on the problem of the evolution of pollination systems.

SELF FERTILIZATION AS A DERIVED CONDITION

A review of the evidence provided by studies of these and other genera shows us that the types which are regularly self fertilized most probably are always derived from cross fertilizing ancestors. The main lines of evidence which point to this conclusion are as follows.

First, in those genera of which species relationships have been studied from the phylogenetic viewpoint, including most of the ones mentioned above, the self fertilizing species appear clearly to be more specialized in morphological characteristics than many of their cross fertilizing relatives. In many instances (Bromus, Hordeum, Medicago, Trifolium) they possess highly specialized mechanisms for seed dispersal. A very high proportion of them have annual life cycles (Stebbins 1950, p. 166), a condition which is generally regarded by morphologists to be derived from the perennial growth habit (Jeffrey 1916, Ames 1939). Furthermore, the annual members of the genera mentioned can nearly always be associated in the sum total of their morphological characteristics with perennial species of the same genus, while among the latter there often exist species of doubtful affinities, which may bridge the gap between genera.

Second, many self fertilizing species possess structures which could have a high selective value only in connection with cross fertilization. In the grasses, both self and cross fertilizing species possess lodicules, small structures at the base of the florets which by intake of water force the florets open, and thereby aid in the dissemination of pollen. In the Leguminosae, both cross and self fertilizing species possess the elaborately constructed papilionate type of flower, the origin of which is almost impossible to imagine except as an adaptation to visits by cross pollinating insects. Equally elaborate flowers are found in self fertilizing species belonging to the Boraginaceae (Amsinckia), Scrophulariaceae (Antirrhinum, Linaria, Mimulus), Valerianaceae (Plectritis), Lobeliaceae (Downingia), and Compositae (Senecio, Microseris, Crepis). A most striking example is that of *Ophrys apifera*. The flowers of this orchid are not only elaborate in form, as are those of most orchids, but in addition possess an intricate color pattern. Nevertheless, they are regularly self pollinated, since the elongation of the staminal column caused the pollen to be extruded and deposited on the stigma before the flower opens.

If we follow the well recognized principle that the origination of elaborated structures, which depend on the interaction of a complex combination of genetic factors, requires much stronger selective pressures than their

maintenance once they have originated, these situations are relatively easily explained. Each of the structures mentioned—the lodicules of grasses, the papilionate flower of legumes, the bilabiate corolla of the Scrophulariaceae, Lobeliaceae, and other families—probably evolved to their present day perfection in a series of cross fertilizing species, in which strong selective pressures existed favoring exchange of genes among individuals and populations of the same species, and reducing the possibility of crossing between species. Once established, these structures became determined by a combination of genes essential for the reproductive activity of the plant. Hence, even after the evolutionary line had changed from cross to self fertilization, so that the selective advantage of these structures had become greatly reduced, the genes responsible for them could not become lost or greatly modified without seriously affecting the vitality or fertility of the plant.

A third reason for regarding self fertilization as a derived condition is that in some groups self fertilizing species or populations have originated in historical times from cross fertilizing species. One example is the cultivated snapdragon, *Antirrhinum majus,* the only self fertilizing representative of a species complex which consists otherwise entirely of self incompatible, cross fertilizing species. It seems likely that in this species self compatibility arose through unconscious artificial selection for fertility in the cultivated plant (Baur, Stubbe). Another example is the series of populations of homostylous plants of the otherwise heterostylous species *Primula vulgaris* found by Crosby (1949) in southern England. This author showed that given equal selective value, homostyles will quickly replace heterostyles in a population. Since the crossover which leads to homostyly, though a rare event, must have occurred many times during the evolutionary history of *P. vulgaris,* the predominance of heterostyly in the species as a whole is best explained on the assumption that most homostyles are at such a great selective disadvantage when they arise that they are quickly eliminated. The recent establishment of homostyles in southern England, therefore, is probably due to recent changes in the environment, in the genetic structure of the species, or in both, which has reversed the usual trend.

The fourth and final reason is that recent genetic work, as summarized by Lewis (1954, 1955), has shown that different genera of the same family or in closely related families which contain self incompatible species usually have a similar genetic basis for the self incompatibility. Thus, the Nicotiana or personate system is general throughout the Solanaceae and Scrophulariaceae, while the sporophytic system appears throughout the Cruciferae and again in the Compositae. Furthermore, studies on artificial induction of mutations in the self incompatibility gene have shown that mutation from one self incompatibility allele to another cannot be induced by radiations, and does not occur spontaneously at a sufficiently high rate to be detected experimentally, while mutations suppressing the activity of

these alleles, that is, from self incompatibility to self compatibility, occur rather frequently.

The origin of self incompatibility systems, therefore, requires the accumulation of a series of rarely occurring genetic events, and must be assumed to have taken place only a few times during the evolutionary history of the angiosperms. Self compatibility, on the other hand, can arise relatively easily, and can be assumed to have originated several times even in the evolution of a single family.

The chief barrier to the origin of self fertilizing lines from cross fertilizing species is that the latter generally contain many deleterious genes in the heterozygous condition, so that they suffer marked inbreeding degeneration. This barrier can, however, be surmounted if the evolution of self fertilization occurs gradually, via a series of stages involving self compatible species which can either be self fertilized or outcrossed. If such species are perennials and have a high reproductive potential, they can tolerate the production of occasional weak or inviable zygotes homozygous for deleterious genes, while the much rarer homozygotes for favorable combinations of alleles, if they find a favorable environmental niche, can from time to time become established and spread rapidly. In my opinion, therefore, facultatively self fertilizing species like *Bromus laevipes* and *B. Orcuttianus* (table I) are necessary intermediate stages between self incompatible species and those which are regularly self fertilized.

REASONS FOR THE ORIGIN OF SELF FERTILIZATION

We now come to one of the central points of my discussion; why should some species have given up cross fertilization, with its attendant advantages of population variability and genetic heterozygosity? In my opinion, there are three reasons, two of them negative and one positive.

In the first place, flowers may resort to self pollination when conditions become unfavorable for crossing. One of the clearest examples of this change was demonstrated by Hagerup (1950, 1951) in the Faero Islands, where the weather is almost constantly rainy. He found that *Ranunculus bulbosus, R. flammula, Caltha palustris, and Narthecium ossifragum* are regularly self pollinated by rain drops, which collect in the flowers, and transport pollen, adsorbed on their surface, from the anthers to the stigma. In the case of *Narthecium ossifragum* an unseasonal period of clear weather resulted in partial sterility through non-pollination of the flowers, even though these are conspicuous, brightly colored and apparently adapted to insect pollination. In continental Europe, Knuth has observed frequent visits to the flowers of Narthecium by bees and other insects, which are rare or absent on the Faroes. Apparently, therefore, *Narthecium ossifragum* is a species which under favorable conditions is adapted to cross pollination by insects, but which has acquired a fertility insurance through a mechanism whereby water can effect self pollination if insects are absent.

Self fertilization as a means of fertility insurance occurs also in plants which are subjected to periodic drought. Chase (1908) has recorded the formation of axillary cleistogamous flowers at the base of the plant in species of Stipa and Danthonia, which inhabit dry places, and in which the usual culm inflorescences may set little seed under conditions of extreme heat or drought. The advantage of the self fertilization which occurs so regularly in the annual species of California and other regions with a climate of the Mediterranean type, may exist partly because of the great annual fluctuation in climate to which these regions are exposed. In unfavorable, dry years conditions favorable for cross pollination might be absent altogether, or present so briefly as to reduce seriously the fertility of species dependent entirely upon this process for reproduction.

Indirect evidence in support of this hypothesis is provided by the fact that in some species which are usually self pollinated, such cross pollination as occurs takes place under unusually favorable conditions. Harlan (1945a) reported that chasmogamous flowers of Bromus with large, exserted anthers, are formed most abundantly during the height of the spring blooming season, and are completely absent when plants are forced to bloom in the autumn, or under any conditions involving low light and a shortage of soil. Stone (1957) showed that in Myosurus the achenes of a flower which are ripe at the time of anthesis are regularly pollinated by the surrounding anthers. If conditions are unfavorable, no more achenes are produced, and the plant is entirely self fertilized. But if the plants are growing vigorously and have abundant water, the receptacle elongates and produces many additional achenes, which may be cross pollinated by insect vectors, since no more pollen is available from the same flower.

A second reason for the establishment of self fertilization is long distance dispersal, as pointed out by Baker (1948, 1953a,b, 1955). He found that in the family Plumbaginaceae, particularly the genera Armeria and Limonium, the species which have dimorphic flowers and are regularly cross pollinated are found chiefly near the Eurasian center of distribution of the family, while the peripheral regions, such as North America and Australia, contain chiefly monomorphic, self fertilizing species. In the sections Candelabra and Farinosae of Primula the centers of distribution in eastern Asia contain almost entirely species with dimorphic flowers and cross pollination while peripheral regions, such as Malaya, the Japanese islands, and North America, contain a majority of self fertilizing, monomorphic species (Ernst 1953). Although Ernst's interpretation of these results does not agree with that of Baker, since he believes that some of the monomorphic types are primitively so, the similarity of the distribution patterns in the two genera suggests a common explanation for them. Baker (1955) gives another example in the genus Coprosma (Rubiaceae) where *C. pumila* Hook., a characteristically dioecious, cross fertilizing species of New Zealand, possesses a monoecious, presumably self fertilizing type on Macquarie Island, in the Antarctic Ocean.

In my study of some genera of the family Gramineae I have found several similar examples. The center of distribution of the genus Bromus is Eurasia, where the greatest morphological diversity occurs. Here are found nearly all of the self incompatible, cross fertilizing species of the genus, particularly *B. inermis, B. erectus,* and their relatives. The species of the New World are nearly all capable of self fertilization, the two known exceptions being *B. auleticus* of temperate South America and *B. Pumpellianus* of northwestern North America. The genus Hordeum is likewise centered in Eurasia, where is found its only self incompatible species, *H. bulbosum.* A most striking example is found in the genus Secale. This small genus occurs almost entirely in southwestern Eurasia. Its most primitive species is *S. montanum* Guss. (sens. lat.), a caespitose perennial which occurs locally in the mountains of the Mediterranean region, from Morocco and Spain east to northwestern Iran (figure 1). It contains several ecotypes which have been regarded by some authors as separate species, but which are completely interfertile when crossed with each other. So far as is known, these ecotypes are all completely self incompatible. The nearest relative of *S. montanum* is *S. africanum* Stapf, which is confined to a single mountain range in South Africa. Morphologically, *S. africanum* is hardly distinguishable from *S. montanum,* and hybrids between the two species have normal meiosis and are fully fertile (Schiemann and Nürnberg-Kruger 1952), so that *S. africanum* could be regarded as a geographically isolated ecotype of *S. montanum.* The most distinctive characteristic of *S. africanum*, however, is its complete self compatibility.

The correlation just mentioned occurs so widely and has such great significance for studies of the origin and migration of genera of flowering plants and probably of other groups, including some animals (Baker 1955), that it deserves recognition as Baker's law. It was logically and reasonably explained by its author on the assumption that accidental long distance dispersal of a single propagule can lead to establishment of a colony only in a species capable of self fertilization. If the type thus established is well adapted to its newly found ecological niche, it can spread throughout the area where these conditions are found, even though its capacity for genetic variation is much reduced.

The reasons given above for the establishment of self fertilization are essentially negative, that is to say, this condition may under some circumstances be tolerated by the population because survival is impossible without it. A third, positive reason for the establishment of self fertilizing populations has been suggested elsewhere (Stebbins 1950, p. 177). This is associated with the colonization of new habitats. A species which normally behaves as a weed, for instance, cannot maintain continuously populations of a constant size, since the habitats which it occupies are temporary. Its populations are constantly being destroyed, and must be built up again in newly available locations. Under such conditions, a high selective advantage is given to a genetic type which can quickly build up large

FIGURE 1. Distribution of the two perennial species of *Secale*, *S. montanum* and *S. africanum*

populations of well adapted individuals as the progeny of one or two initial colonizers. This ability depends not only upon a high reproductive capacity, but also upon the assurance that the descendants of a single well-adapted initial colonizer will be equally well-adapted to these same conditions, that is, will have adaptive properties similar to their parent. The genetic diversity which results from cross fertilization and its attendant

heterozygosity, though undoubtedly of great value in enabling populations to evolve in response to a gradually changing environment, would be a distinct disadvantage in the particular situation mentioned above, since a heterozygous initial colonizer would be certain to produce segregating offspring with different adaptive properties. Given an initial adaptive advantage, therefore, a homozygous individual belonging to a self fertilizing line would be most likely to transmit this advantage to its descendants.

There are several reasons for believing that this positive selective value has often aided in the establishment of self fertilizing types. In the first place, self fertilization is found in many field weeds adapted to highly favorable climates, which therefore have never been exposed to severe environments. Some well known examples are *Senecio vulgaris* (Haskell 1953), *Stellaria media, Galeopsis* spp., *Vicia* spp. and *Sonchus oleraceus.* Furthermore, these species occur at or near the centers of distribution for their genera, and there is no reason for believing that they have ever been subjected to long distance dispersal. Secondly, many species of annuals, even those adapted to dry regions, maintain their populations in spite of enforced cross pollination because of self incompatibility. Examples may be cited in Crepis (Babcock 1947), Layia, and Holocarpha (Clausen 1951). In at least some of these species a temporary genetic constancy, comparable to that acquired through self fertilization, may result from increased linkage, holding together adaptive blocks of genes. This is suggested by presence in these species of reduced chromosome numbers, reduced chiasma frequencies, or both.

These considerations lead to the following general hypothesis. Plant populations adapted to certain types of temporary habitats, particularly annual weeds and other colonizers of newly available situations, possess a considerable selective advantage if they remain constant genetically for periods of many generations, but still can produce occasional bursts of genetic variability. The commonest genetic system which brings about such cycles of alternating variability and constancy is that of predominant self fertilization, with the capacity for occasional outcrossing being nevertheless retained.

THE EFFECT OF SELF FERTILIZATION ON VARIABILITY IN NATURAL POPULATIONS

There are now several analyses available of natural populations belonging to predominantly self fertilizing species. Examples are *Bromus carinatus* (Harlan 1945b), *Senecio vulgaris* (Haskell 1953), *Gilia clivorum* (Grant 1954a), *Microseris Douglasii* (Chambers 1955), *Clarkia purpurea* (Lewis and Lewis 1955 pp. 300-308), Amsinckia, sect. muricatae (Ray and Chisaki 1957), and *Myosurus* spp. (Stone 1957). The results of these analyses agree in their general features, and give us the following conception of the variation pattern within species of this type. The natural population consists of several morphological types, each of which is represented by many

similar or identical individuals. These types can be recognized by distinctive morphological characteristics, so that the population appears like a cluster of closely related but separate microspecies. In addition to these dominant morphological types, there may exist also several other recognizable types which are represented by only one or two individuals.

If seeds are collected from a single plant of one of the dominant types, the progeny are most often found to be entirely uniform, and breed true for an indefinite number of generations, indicating that the original plant was completely homozygous. In some instances, as in certain families of *Senecio vulgaris* (Haskell 1953), the variation decreased slightly in the second and third generations of automatic selfing under artificial isolation, indicating a slight degree of heterozygosity for the original plant.

When different morphological types belonging to the same or adjacent populations are intercrossed, the F_1 hybrids may be completely fertile, as in Senecio, Gilia, Microseris, Clarkia, and Myosurus, or they may show a greater or lesser degree of sterility, as in *Bromus carinatus* (Stebbins and Tobgy 1944), *Galeopsis tetrahit* (Muntzing 1938), *Elymus glaucus* (Snyder 1950, 1951), and *Trifolium subterraneum* (Morley, Brock, and Davern 1956). The latter examples will be discussed below in connection with the species problem in self fertilizing groups.

From the information presented above, the following pattern of reproductive behavior has been reconstructed by several of the above mentioned workers for the groups concerned. Each successful biotype maintains itself as a constant, genetically homozygous pure line for a large number of generations and is represented by hundreds or thousands of individuals. It is normally isolated by self fertilization from other biotypes of the same species with which it grows sympatrically. Occasionally, however, accidental crossing between biotypes may take place, resulting in vigorous F_1 hybrids. In some species these hybrids are more vigorous than their parents (*Galeopsis tetrahit,* Hagberg 1948) while in other species they are not (*Gilia clivorum,* Grant 1954a). The selfed progeny of these hybrids show a wide range of segregation in the F_2 generation, which continues to a diminishing degree in 7 or 8 subsequent generations. The great majority of these segregating individuals are more poorly adapted than the original parental types, and so are eliminated by natural selection. A few of them, however, are retained in the population and so form the progenitors of new lines. Although no data are available on this point, we might expect that when a self fertilizing species is invading a new habitat, a larger number of new lines might become established than in the case of a species population which is maintaining itself in the same habitat.

The model of breeding structure just presented resembles in its essentials the type of population structure which Wright (1931) postulated as being best equipped for continuous evolution in either a stable or a varying environment. He pictured a population of intermediate size, divided into many subunits, each of them separated spatially from each other, but capa-

ble of occasional gene exchange through migration between subunits. Because of this migration, the subunits share a common gene pool, and valuable new mutations can spread through the entire population through the action of hybridization and selection. On the other hand, inbreeding within subunits permits the establishment to a certain degree of chance variants, and enables different subunits to occupy slightly different adaptive peaks.

In the self fertilizing species mentioned above, the breeding structure postulated by Wright exists in a modified form. The subunits of the population are isolated from each other not spatially, but by their breeding behavior. Different biotypes occupy the same or very similar ecological niches, and presumably represent slightly different adaptive peaks. Gene exchange occurs as a result not of migration, but through accidental breakdown of the normal mechanism for self fertilization. The great abundance of such species as *Bromus carinatus, Senecio vulgaris,* and *Clarkia purpurea* is sufficient evidence that this type of population structure may be highly successful.

This type of breeding behavior, which appears highly anomalous to biosystematists familiar only with cross fertilizing populations, justifies a different type of taxonomic treatment. The clusters of biotypes which represent extreme morphological and ecological variants of these species may occur sympatrically over wide areas of geographic distribution, but may nevertheless remain more or less distinct from each other because of a low incidence of crossing, in spite of their potential interfertility. They thus possess the genetic as well as the morphological characteristics of different subspecies. It is, therefore, not surprising and entirely justifiable that Chambers (1955) has recognized subspecies with sympatric areas of distribution in *Microseris Douglasii,* and Lewis and Lewis (1955) have done the same in *Clarkia purpurea.*

The above discussion applies clearly only to those species which are facultatively self fertilized, and are capable of occasional outcrossing. If a species is entirely self fertilized, it should consist of a series of completely homozygous pure lines, each of them sharply distinct from the others. This is apparently true of the strains of *Trifolium subterraneum* which have been introduced into Australia (Donald and Smith 1937, Aitken and Drake 1941, Davies 1951), but personal observation of the variability of this species as it grows in Algeria, where it is native, suggests that occasional outcrossing probably takes place in that region. So far as I am aware, no species of plant has been studied intensively throughout its entire range of distribution and has been found to be exclusively self pollinated everywhere.

THE ORIGIN OF SPECIES IN SELF FERTILIZING GROUPS

Self fertilization may affect strongly the way in which species originate, by altering the mode of origin of barriers of hybrid sterility. This can be manifest both at the homoploid level, and through the formation of species

by means of polyploidy. At the homoploid level, the effect is brought about through differences in the mode of segregation of genetic factors, either genes or small translocated chromosomal segments, in the progeny of highly but not completely sterile interspecific hybrids. Empirical data in a number of genera (Erophila, Bromus, Galeopsis, Elymus, Trifolium, Phaseolus, Amsinckia) indicate that self fertilizing groups are more likely to be divided into a number of microspecies, or of populations which are partly isolated from each other through varying degrees of sterility in their F_1 hybrids, than are cross fertilizing groups. This phenomenon was ascribed by Anderson (1953) to the differential effects of introgressive hybridization, and the writer has obtained strong evidence in the genus Elymus that this viewpoint is essentially correct (Stebbins 1957b).

A genetical model which will illustrate the difference between cross and self fertilizers in this respect is as follows. Assume the existence in related species of sets of complementary gametic lethals. These could be either genes or small translocated segments. If the two parents of a cross should differ from each other in respect to only one such pair, that is, AAbb × aaBB, the heterozygous F_1, AaBb, would be semisterile if unfavorable interaction between *A* and *B* or between *a* and *b* should cause the death of any gamete which differed in its constitution from the parental gametes (that is, gametes of the constitution *AB* and *ab*). Selfing such a semisterile hybrid would give in F_2 the parental types and the heterozygote in the ratio of 1 : 2 : 1, while backcrossing would give, in the 1 : 1 ratio, the heterozygote and the recurrent parental type. In this simplest example, therefore, new lines isolated from both of the original parents could not arise.

The result, however, is completely different if the parents differ with respect to two or more pairs of complementary gametic lethals, each pair of which segregates and produces its effects independently of the others. The simplest possible model is as follows.

Parents: *AAbbCCdd* × *aaBBccDD*.

F_1 hybrid: *AaBbCcDd*.

Viable gametes of F_1 (forming 25% of the total): *AbCd, aBcD, AbcD, aBCd.*

In this example, the effects of self fertilizing the F_1 hybrid would be entirely different from those of backcrossing it to its original parents, as would be most likely to happen if one or a few such hybrids should exist in a population of outcrossing plants. Self fertilization would result in the production of a number of semisterile progeny, heterozygous for one or both of the complementary pairs, plus four types of fertile homozygotes, *AAbbCCdd*, *aaBBccDD*, *AAbbccDD*, and *aaBBCCdd*. The latter two would form semisterile hybrids with both of the original parents as well as with each other. Each of these types, therefore, could be the origin of a genetic line partly isolated from every other genotype in the species and, therefore, of a new microspecies.

If backcrossing took place, the only fertile homozygotes which could be produced would resemble the recurrent parent in their genetic constitution.

The various types of heterozygotes which would result in such backcrossing would be eliminated from the population by natural selection because of their partial sterility, unless the heterozygosity, either for the factors themselves or for other genes linked to them, should confer a selective advantage on the individuals concerned.

The model presented above is undoubtedly an oversimplification, since segregation for fertility in later generations of highly but not completely sterile hybrids of Bromus, Oryza, and other genera, indicates that many different pairs of factors contribute to this sterility (Stebbins 1958). The point must be emphasized, however, that the difference between the results of selfing and of back- or outcrossing becomes progressively greater, the larger is the number of sterility factors by which the parents differ. If one assumes strictly additive effects on fertility, and independent segregation of different factor pairs, then the ratio of homozygotes with new combinations, partly isolated from both parents, to the total number of fertile homozygous types which can result from self fertilizing the F_1 plant, as well as some of its heterozygous F_2 progeny, is $\frac{2^n - 2}{2^n}$, where n is the number of independently segregating sets of factors. One can see from this computation that even if the parents should differ by a relatively small number of such sets of factors, say 5 or 6, the great majority of fertile F_2 types which could be produced by selfing the F_1 would represent newly isolated lines and potential new microspecies. In cross fertilizing species, on the other hand, no matter how great is the number of such sets of factors which differentiate a given set of parents, partly isolated homozygotes can appear only through the relatively infrequent occurrence of crossing between similar heterozygotes. In cross fertilizers, therefore, as Anderson (1953) has indicated, hybridization and introgression between species capable of forming F_1 hybrids with a low degree of fertility would most likely inject into the populations of one of the original species chromosomal segments derived from the other. If these segments persisted because of positive natural selection, they would cause partial sterility in hybrids between certain individuals of the same or neighboring populations, as has been found by Clausen (1951) in Layia, Lewis (1953) in Clarkia, and Grant (1954b) in Gilia, but they would not generally be expected to initiate new, isolated lines.

Another difference between predominantly self fertilizing species and those with obligate cross fertilization lies in the origin of new species through polyploidy. Grant (1956) has pointed out that in many groups which consist of annual or biennial species, e.g., Layia, Madia, most sections of Gilia, Amsinckia (Ray and Chisaki 1957), the self fertilizing species may contain several allopolyploids, while those with obligate cross fertilization are strictly diploid. He ascribes this condition to the difficulty of establishing polyploids from sterile interspecific hybrids, either through gametic or somatic doubling, during the short span of life possessed by

these plants, plus the fact that gametic doubling can effectively give rise to polyploidy in a cross fertilizing plant only if two neighboring plants produce simultaneously unreduced gametes, and these rare gametes happen to unite. Such a sequence of rare events has such a low range of probability that it almost never occurs.

A review of all polyploids known to me in self fertilizing groups, both annuals and perennials, reveals the fact that they are uniformly allopolyploid with respect to their cytogenetic behavior, the great majority of them forming exclusively bivalents at meiosis. In a few instances, such as *Agropyron agroelymoides* (Hunziker 1953) and *Oryza Eichingeri* (Shama Rao and Seetharaman 1955) the self fertilizing species can form some multivalents, and partial pairing between their component genomes occurs in interspecific hybrids, but such examples are exceptional. All the examples of complete or nearly complete autopolyploidy, such as *Dactylis glomerata, Agropyron cristatum, Hordeum bulbosum, Phleum pratense, Medicago sativa, Biscutella laevigata, Tradescantia* spp., and *Solanum tuberosum,* are in species which are extensively or exclusively outcrossed under natural conditions.

This situation appears at first sight to be somewhat paradoxical. One might expect that in predominantly self fertilizing organisms both interspecific hybrids and the allopolyploids derived from them would be less common than in cross fertilizers, and that autopolyploidy would occur relatively frequently. We can explain it satisfactorily, however, if we assume that the frequency of polyploid types within a particular group depends not at all upon the frequency with which they originate, but entirely upon the ability of newly formed polyploids to survive and spread because of superior adaptive properties. On the basis of this assumption, three reasons can be suggested for the scarcity or absence in self fertilizing groups of autopolyploids or segmental allopolyploids.

First, as I have suggested elsewhere (Stebbins 1956), autopolyploidy probably benefits the population not primarily because of the superior qualities conferred by the increase in chromosome number *per se,* but by its buffering effect on certain heterozygous gene combinations which promote either a general hybrid vigor or certain specific adaptive properties. The principal evidence in favor of this hypothesis is that many of those natural autopolyploids which have been analyzed genetically prove to be highly heterozygous, and to contain genes derived from two or more different diploid ecotypes or ecospecies. Such heterozygous genetic conditions could be maintained in populations of cross fertilizers by means of natural selection, but not in self fertilizers, which would eventually become essentially homozygous even as tetraploids.

Second, as was mentioned earlier in this article, most self fertilizers are annuals. They are, therefore, ill adapted to passing through the bottleneck of partial sterility which has been found to exist in nearly all "raw" autopolyploids which have been artificially synthesized. We might expect,

therefore, that the great majority of autopolyploids which appear spontaneously in populations of self fertilizers would be quickly eliminated because of their partial sterility and other inadaptive properties resulting from genetic unbalance. In cross fertilizing perennials, on the other hand, a certain degree of sterility might be tolerated in the raw autopolyploid, and its descendants might acquire from other genotypes modifiers which would restore the genetic balance.

Finally, the differentiation of the genomes from a partly autopolyploid or segmental allopolyploid condition to one more nearly approaching typical allopolyploidy could progress more rapidly in self fertilizing than in cross fertilizing species. Such differentiation could take place most easily through the medium of translocations between non-homologous chromosomes belonging to the same genome, but would be effective only if the plants concerned became homozygous for such translocations. This might take place relatively easily in self fertilizers, but would be much more difficult in cross fertilizers, or might even be prevented in the latter by the presence of recessive deleterious genes on the chromosomes concerned.

The above discussion does not imply that allopolyploid species always occur with a higher frequency in self fertilizing than in cross fertilizing species. In most genera containing many perennial species the reverse is true, and the total incidence of polyploidy in self fertilizers is considerably lower than it is in cross fertilizers. This situation exists in such genera as Trifolium and Medicago, in which polyploids are found among the cross fertilizing species, but are completely absent from the self fertilizers.

THE EVOLUTIONARY POSSIBILITIES OF SELF FERTILIZING SPECIES

From the theoretical point of view, we should expect that self fertilization, by reducing the size of the gene pool upon which a population can rely for the formation of new combinations to fit changing environmental conditions, would reduce markedly the evolutionary potentialities of those lines which adopted it. Actually, this reduction cannot be estimated directly, since the type of plants which possess predominant self fertilization, annuals and some herbaceous perennials, have left almost no fossil record by means of which their evolutionary progress could be traced. Indirect evidence on this point can, however, be obtained from the extent of diversity in the existing self fertilizing groups and their phylogenetic affinities.

Such studies tell us that few if any large genera of angiosperms consist entirely of self fertilizing species, and in each genus containing both selfers and cross fertilizers the latter, as stated above, invariably possess characteristics which indicate that they are more primitive than the former. One can safely make the assumption, therefore, that self fertilization slows down evolutionary progress in the flowering plants to such an extent that groups given over to it can rarely if ever evolve new genera, and can under

no circumstances give rise to new families. On the other hand, many of the self fertilizing groups have proliferated a large number of species, and some of them, like the grass genera Danthonia and Oryza, have patterns of geographic distribution which suggest that they are relatively old. In one sense, therefore, self fertilization is an evolutionary "blind alley", since it apparently closes the door to the elaboration of radically new adaptive devices. On the other hand, a group of species may travel a long way down this "alley" by evolving new variations on the theme laid down for them by their cross fertilizing ancestors. Furthermore, the adoption of predominant self fertilization is by no means the prelude to evolutionary extinction, since some self fertilizing groups appear to have existed for several geological epochs, and may even have outlasted many of their cross fertilizing relatives.

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GENETIC ASPECTS OF ANURAN POPULATIONS[1]

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An analysis of the phenotypes of a local population of an anuran species usually reveals considerable individual variation. The individuals vary in absolute body size, proportions of various body components, distribution of dorsal spots and warts, degree of ventral markings, color pattern, and other qualitative and quantitative characters. However, knowledge of the bases and extent of the variation in a given deme (gamodeme of Gilmour and Gregor, 1939) is limited.

Character analyses have been undertaken principally to clarify the taxonomy of populations of frogs and toads. Taxonomic judgments rarely have been complemented by inquiries into the genetic nature or adaptive significance of the characters chosen. Similarly, studies on the geographic distribution and broader aspects of the ecology of anuran species have far outnumbered those on the attributes (collectively, the structure) of local populations. Consideration is given herein to selected researches that have yielded information on the genetic composition and structure of anuran populations.

POLYMORPHISM

In certain anuran populations, pigmentation patterns have been detected that appear to be discontinuously variable, that is, consist of two or more discrete types. The rarest of the contrasting types occurs too frequently in natural populations to be maintained solely by mutation pressure. Little is known of the forces regulating the polymorphic state in nature; the studies have thus far centered on ascertaining the mode of inheritance of the different patterns.

Two main types of dorsal pattern, striped and spotted, are distinguishable in *Discoglossus pictus pictus,* a toad that inhabits Tunisia, Algeria, Morocco, Spain, and the southwest corner of France (Bruce and Parkes, 1947; Lantz, 1947). Lantz's breeding data suggest that the striped pattern is a simple Mendelian dominant to the spotted variety. As far as known, the two types occur together, in approximately equal numbers, throughout the distribution range. However, the striped form is absent in populations

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of *D. pictus sardus,* a subspecies restricted to certain islands in the western Mediterranean. A comparable dimorphism of dorsal pattern, a presence or absence of a dorso-median stripe, occurs in individuals of *Rana limnocharis.* Different populations in Japan contain varying proportions of the striped form. The presence of a dorso-median stripe is apparently the result of a single dominant gene (Moriwaki, 1953). Moriya (1952) investigated the dimorphism of ventral pigmentation in the pond frog, *Rana nigromaculata nigromaculata.* The presence of black pigment on the ventral surface is recessive to the more common unpigmented condition.

Terrestrial frogs of the genus Eleutherodactylus exhibit a high degree of polymorphism in dorsal pigment patterns. Goin (1947, 1950, 1954) has demonstrated a genetic basis for some of the polymorphic types. One particular group, *E. ricordi planirostris* (greenhouse frog), is widespread in Cuba and the Bahama Islands, and recently has become established in Florida. Goin surmises that the greenhouse frog was introduced into Florida by means of clutches of eggs accidentally included in shipments of plants from Cuba. The present populations in Florida consist of small, isolated colonies.

The greenhouse frog possesses either of two dorsal patterns, striped and mottled. The distinction between them is unifactorial, striped being a simple dominant. The proportions of the two patterns vary in different colonies in Florida, although the recessive mottled type predominates in the majority of the populations. A single clutch of eggs could initiate a colony, which, in turn, would establish at the outset a given pattern or proportion of patterns. As the effective breeding sizes of the local populations are small, subsequent fluctuations in gene frequencies could be due to accidents of sampling ("Sewall Wright effect"). Evidence that the dimorphism is maintained by a balance of selective forces is lacking. If balanced polymorphism is involved, the phenotypic similarity of the heterozygote and dominant homozygote renders improbable the adaptive superiority of the former due to its externally visible dorsal pattern.

Two pigment variants of the common leopard frog, *Rana pipiens,* have been found in certain populations from the midwestern area of the United States, particularly Minnesota. The nonspotted variety, originally described by Weed (1922) as a new species, *R. burnsi,* was demonstrated by Moore (1942) to be a simple Mendelian dominant to the common spotted pattern. A mottled variety, likewise designated by Weed (*op. cit.*) as a new species, *R. kandiyohi,* was shown by Volpe (1955a) to be also a simple dominant mutant of the common leopard frog. Results of breeding tests indicate, among other possibilities, that the genes responsible for the pigment patterns are allelic (Volpe, 1956a). The nonspotted or burnsi gene has been designated C^B; the mottled or kandiyohi allele, C^K; and the common spotted or pipiens allele, c^+. Recent results (Volpe, unpublished) reveal that the mutant genes lack dominance with respect to each other; the compound or double dominant, $C^K C^B$, combines the characteristics of the

two mutant forms.[3] It is now possible to distinguish a "mottled" individual carrying the burnsi gene (C^KC^B) from a mottled form carrying the common spotted allele (C^Kc^+). This distinction was not evident in earlier work (Volpe, 1956a).

Estimates of the relative frequency of the nonspotted or burnsi variant range from 0.4 to 10.0 percent of leopard frog populations in Minnesota (Swanson, 1935; Moore, 1942; Breckenridge, 1944). The proportion of the mottled or kandiyohi mutant varies from 1.0 to 2.0 percent (Breckenridge, *op. cit.*; Volpe, 1955a). All three forms are known to occur in certain localities in Minnesota; in some areas, only one or the other mutant type is associated with the common spotted leopard frog (Breckenridge, *op. cit.*). The double dominant, C^KC^B, if present in nature, apparently has escaped detection. The dominant genes may be advantageous by themselves but disadvantageous when they interact with each other.

A strikingly similar polymorphism in populations of the cricket frog, *Acris gryllus,* in central Texas has been described by Pyburn (1956). The color of the vertebral stripe may be red, green, a combination of the two, or gray. It appears likely that the varieties depend upon a series of multiple alleles; the "green" and "red" alleles exhibit no mutual dominance, but both are dominant over the "gray" gene. Although the frequencies vary in different local populations, the recessive gray form is invariably the predominant type. The compound, red-green, is extremely rare in natural populations and shows reduced viability in laboratory cultures.

Both of the foregoing situations appear to parallel other cases of polymorphism characterized by an abundant "universal recessive" associated with a large number of relatively rare dominant forms, for example, in the grouse locust *Apotettix eurycephalus* and *Paratettix texanus,* studied by Nabours (1917, 1925), or the fish *Lebistes reticulatus,* investigated by Winge (1927). To account for the excessive amount of close linkage and apparent allelism of many of the dominant genes observed in these organisms, Haldane (1920) and Demerec (1928) suggested that the chromosomes containing the dominant genes do not segregate independently but are transmitted as a group. Haldane (1930) later inferred that the close association of chromosomes is attributable to sectional translocations, and he further surmised that the dominant genotypes are not due to point mutations but to duplications of the translocated segments. The essential point, as contended by Fisher (1930), is that each dominant gene (possibly duplicated gene) may be located in a chromosomal section, in which crossing over is effectively suppressed. Consequently, the rarer dominants may be maintained as polymorphic forms by the selective advantage conferred upon the heterozygotes by genes located in the permanent chromosomal tracts. Reviewing Nabours' data on *A. eurycephalus,* Fisher (*op. cit.*)

[3]This finding has been established independently in another laboratory. Dr. Rodolfo Ruibal at the University of California (Riverside) informed me that Mr. Steven Anderson, working under his direction, has likewise demonstrated the codominance of the mutant alleles.

found a significant deficiency of the dominant homozygotes. Subsequently, Fisher (1939) concluded, from analyses of samples of *P. texanus* collected in nature, that the double dominant individuals were at a great selective disadvantage to those carrying a single dominant gene.

Whether or not the data on the genetics of leopard frogs or cricket frogs are interpretable along similar lines awaits the accumulation of considerably more information than is now available. A balanced polymorphic system may be operative, the alleles being maintained by the adaptive superiority of the heterozygotes. The gene frequencies, however, may be modified by local differences in selection pressures, chance sampling of gametes when the breeding size is reduced at times to small numbers, and interpopulation migration. Critical experimental testing of the comparative viability or fertility of the heterozygotes and corresponding homozygotes is presently hampered by the lack of a standard technique for rearing a large population of tadpoles through and beyond metamorphosis. Many investigators have experienced unexplained mortality in cultures of tadpoles. Inviability among the larvae or transformed young may reflect more imperfect technique than differential mortality. Moreover, frogs, and to a lesser extent, toads are difficult to rear to sexual maturity, severely restricting experimentation beyond the F_1. Some investigators (A. P. Blair, 1941b, 1946; Ting, 1948; Kawamura, 1950; Moriya, 1951; Thornton, 1955; Pyburn, 1956) have had limited success in rearing anurans to sexual maturity.

Not all major genic differences analysed in anurans are associated with polymorphism. A summary of major variants that are seldom seen has been prepared by Rostand (1951).

QUANTITATIVE AND ADAPTIVE CHARACTERS

The differences between individuals and between populations are most often of the graded, quantitative type. Systematists have tacitly assumed that observable quantitative differences between populations are genetically fixed. Investigations designed to reveal the heritable portion of the variations rarely have been undertaken.

Measurable body components, such as, body length, foot length, and head width, are highly subject to individual (inherent), ontogenetic, sexual, and environmental variation. No method has been devised yet to delimit accurately different age groups in a natural population. Even when measurements of two morphological variables are translated into a ratio, for example, the proportion between body length and foot length, the effects of ontogeny or other influences are not obviated. Thus, two population samples that differ in various proportions of body parts may reflect, among other things, an undetectable age imbalance rather than actual genetic differences. The argument may be advanced that the growth of body parts of an anuran after sexual maturity is slight and is affected little, if at all, by environmental factors. This supposition is based more on faith than actual demonstration.

In essence, interpopulation crosses should be performed under controlled conditions to ascertain whether or not the apparent quantitative differences between populations are manifested by the respective progeny. Likewise, the manner in which the continuous characters segregate in interpopulation hybrid offspring should be determined. Ruibal (1957) applied such procedures in dealing with variations in snout shape in populations of the leopard frog, *Rana pipiens.* The shape of the snout was expressed as a ratio of two measurable linear components, head height divided by head length (H/L). Ruibal demonstrated a north-south, as well as an altitudinal, cline in snout shape, the northern or highland populations having a higher H/L ratio or shorter, blunter snout than southern or lowland populations. Progeny of crosses between highland and lowland populations reared under the same laboratory conditions reflected the differences of the respective parental populations. Hybrid offspring were variously intermediate in snout shape. Polygenic control of snout shape was indicated by the types of distribution observed in the parental and hybrid offspring. Schmidt (1938), A. P. Blair (1941b), Inger (1950), Smith and Smith (1952), and Mecham (1954), among others, have demonstrated goegraphical clines in certain metric characters in other anuran species, but the extent to which the interpopulation variations are attributable to differences in genotype was not determined.

The question arises as to the adaptive value of many of the morphological characters distinguishing the populations. What is the adaptive significance, for example, of changes in the relative size of the parotoid gland in populations of Bufo species or in the degree of differentiation of the dorsolateral folds in populations of Rana species? They may be presumed to be associated with more fundamental adaptive physiological changes.

Some comprehension of the adaptive value of characters in relation to the habitat of the species has been derived by exploring the behavior of individuals under known experimental conditions. Gosner and Black (1957) demonstrated that the embryonic tolerance for different pH conditions varied in eleven anuran species distributed in the pine barrens and adjacent areas of New Jersey. Embryos of those species inhabiting the pine barrens were able to tolerate strongly acid water, characteristic of the bogs and swamps of the region. The data suggest that low pH may be one of the factors limiting the occurence of various species in the pine barren region. Among recent workers, Rey (1937), Thorson and Svihla (1943), and Thorson (1955) have determined experimentally the vital limits of water loss of several anuran species. The extent of tolerance to water loss is correlated with the degree of terrestrialism. Moore (1949a,b and earlier papers) followed by his students (Volpe, 1953, 1954, 1957a,b; Ruibal, 1955) have dealt with the effects of temperature on the development of embryos of North and Central American species of frogs and toads. These studies have revealed that a number of embryonic characteristics, such as rate of

development and temperature tolerance, are closely correlated with the geographical distribution and breeding habits of the species. The several aforementioned papers (see also Tamini, 1947; Douglas, 1948) should be consulted for details.

The embryos in these studies were tested over a wide range of constant temperatures to reveal, under a set of known experimental conditions and specific criteria, the endurable limits of tolerance of the embryos. What has been realized experimentally is the potential norm of reaction to temperature of the gene complex characteristic of members of each species. The demonstration that the gene complex of each species responded differently to experimental temperatures indicates that a given reaction range has been established by selective action. The implication is that a particular response to temperature is of adaptive value. Moreover, data by Moore (1946, 1947, 1950) suggest that interpopulation differences in embryonic temperature adaptations may be the basis for the hybrid defects observed in certain intraspecific crosses.

It is recognized that the results of experiments under constant temperatures are not equivalent to the outcome under fluctuating temperatures in nature. Short or intermittent exposures to extreme temperatures in nature may have little or no effects on developmental processes, whereas continuous exposure as in the experimental situation may be deleterious or lethal. Nevertheless, it is possible on the basis of laboratory tests under controlled conditions to predict approximately the behavior of individuals under natural conditions. For example, results of experimentation (Moore, 1949b) indicate that cold-adapted species compensate for low temperatures of their environment by a rapid developmental rate. This has been corroborated by field observations. Karlstrom (1956) compared the development of embryos of *Bufo boreas* in their natural environment at 4,000 feet in Yosemite valley with those of *B. canorus* at 9,900 feet in Tioga Pass. Water temperatures were recorded at the sites in which the embryos of the respective species developed. Tadpoles of *B. canorus* transformed into young toads within the same period of time (47 ± 5 days) as those of *B. boreas* (46 ± 5 days), even though the former developed in colder waters.

A rapid rate of development is also correlated with other environmental factors. Species that deposit their eggs in shallow rain pools that evaporate quickly possess a more rapid rate of embryonic development than those that breed in permanent bodies of water (Volpe, 1957a).

POPULATION STRUCTURE

The conspicuous and concealed variability in a species population is affected by such phenomena as home range, territorial behavior, seasonal fluctuation in population size, effective breeding size, sex ratio, reproductive behavior and potential, growth and longevity, differential survival of progeny, and dispersal. There are few precise and detailed studies of these factors in natural populations. In the literature may be found an ar-

ray of desultory field observations, much of which cannot be integrated into a comprehensive picture. Statistically significant information on population dynamics can be obtained only by carefully executed, long range studies on selected local populations.

A standard technique for population studies is the marking, releasing, and recapturing of constituent individuals of a population. The means of marking frogs for future identification comprise the use of waterproof paper tags (Breder, Breder, and Redmond, 1927; Savage, 1934), the use of metal jaw tags (Raney, 1940), toe clipping (A. P. Blair, 1942, 1943, 1947a; Bogert, 1947; Martof, 1953a,b; Fitch, 1956a; Jameson, 1955, 1956, 1957; and others) and the use of radioactive lead capsules containing Co^{60} (Karlstrom, 1956). Woodbury (1956) has reviewed the techniques that have been employed.

A limited home range has been demonstrated for some species populations; a given individual lives within the boundaries of a narrowly circumscribed area, apparently familiar to it, and only rarely enters an adjacent terrain. Most marked individuals of *Microhyla olivacea* (= *Gastrophryne olivacea*) in a Kansas population were recovered at varied time intervals within a few yards to 400 feet of the original site of capture (Fitch, 1956a). Exceptionally long movements (up to 2000 feet), representing either a shift in home area or motivated by reproductive urge, were made by a few individuals. Males were more vagrant than females, as had been shown also by Anderson (1954) for *Microhyla carolinensis* populations in Louisiana. Similarly, the cliff frogs (*Syrrhophus marnocki*) in local populations in Texas studied by Jameson (1955) were found to confine their activities to restricted areas. Martof (1953b) found evidence of a well-developed homing behavior in the green frog, *Rana clamitans*. The most extensive movements involved migrations to and from the breeding sites. Juszczyk (1951) reported comparable findings for the European frog, *Rana esculenta*. The same frogs were repeatedly recaptured at particular sites for a period of several years. In the spring the frogs migrated from the stream where they hibernated to adjacent ponds, selecting particular ponds for breeding purposes. After the mating period, the frogs were observed to return to familiar stations in the stream, in preparation for hibernation in the autumn. Bogert (1947) demonstrated that adults of *Bufo terrestris* returned to a favored home site, even after having been removed as much as a mile from familiar territory. The tendency for individuals to remain in closely circumscribed home areas may be a more common phenomenon in anurans than has been generally recognized.

Local differentiation is enhanced by the resident behavior of the adults. Occasional immigration would tend to preserve the plasticity of the local units. Most of the interpopulation exchange apparently is effected through the exploratory movements of young individuals, seeking suitable habitats for residence. The extent of interpopulation dispersion depends, among other things, upon the relative densities of the local units and the degree

of effectiveness of the intervening ecological barriers. There is, however, little information available on the rate and extent of dispersal in anuran populations. Jameson (1955) demonstrated that individuals of *Syrrhophus marnocki* will disperse, at least under induced circumstances, into areas of low population density. When a large percentage of cliff frogs was removed from one part of an experimental plot, numerous individuals moved into the depopulated habitat. In another instance, marked cliff frogs were added to a population of high density and none were found to establish residence in the already well-populated habitat.

The unique breeding habits of salientians affect considerably the nature and extent of local differentiation. The pattern of breeding differs among species and among populations of the same species in different ecological situations. The breeding season is sharply delineated in certain climatic regions, being influenced primarily by temperature (defined as the mesic pattern by Bragg, 1945, 1950). At Ithaca, New York, Wright (1914) was able to arrange the local species of frogs in a definite sequence, according to the onset and duration of the breeding season. A restricted breeding season is not characteristic of species inhabiting dry regions; rainfall replaces temperature as the primary stimulus for spawning. In a sense, species with the xeric pattern (Bragg, *op. cit.*) do not have a breeding *season* but rather breeding *periods*. Each heavy rainfall initiates a new cycle of migration and mating.

The xerically-adapted species are of particular interest. For example, the breeding activity of *Microbyla carolinensis* occurs in Louisiana from the latter part of April to the middle of September (Anderson, 1954). In any given local population, several well-spaced spawning periods may occur. To what extent may each of the several breeding units be considered as parts of the same breeding community? Anderson (*op. cit.*) implied the possibility of considerable subdifferentiation or even reproductive fragmentation of a single population through inherent differences in time of breeding. It seems likely, however, that the apparent reproductive discontinuity between the breeding units is bridged by males returning to breed at different periods in the same season. In the closely related *Microbyla olivacea,* males have been reported to mate several times in one season (Fitch, 1956a). Moreover, the distribution of heavy rains varies from one year to the next. Closely spaced rainfalls in a given year would tend to amalgamate the breeding periods and swamp any tendency toward intensive subdifferentiation. The question remains, however, as to the factors that cause one female to deposit her eggs, for instance, in April, and another female, in September. The possibility exists that a female is capable of spawning more than once in a single season (Bragg, 1944). Little attention has been focussed on the relation of the gonadal cycle to the breeding patterns of anurans in different climatic regions (see G. Cei, 1944; J. M. Cei, 1949; Caruso, 1949; Fisher and Richards, 1950; Anderson, 1954).

The effective breeding size is much smaller than the apparent number of individuals in a swarm. The offspring are derived from a small percentage

of the total local population. Many investigators have noted the disproportionate sex ratio favoring males. A. P. Blair (1943) found that males outnumbered the females by a ratio of 6:1 in breeding aggregations of *Bufo americanus* in northeastern Oklahoma. Such a high ratio is not atypical; indeed, it may be as great as 10:1 (Karlstrom, 1956). The samples or counts are biassed to some extent by the proclivity of females to leave the breeding site after spawning whereas males tend to remain behind. Several workers have concluded that not all females breed each year. Collections of toads in the non-breeding periods approach the expected 1:1 ratio (Miller, 1909; Bragg, 1940; A. P. Blair, 1943).

Males generally precede females to the breeding pools; the obvious surmise is that the mating calls of the males attract the females. Critical evidence of the attracting power of the sex call is lacking. Indeed, Cummins (1920) and Savage (1935) minimize the importance of voice in initiating or directing the migration to the breeding site. Nor has it been demonstrated that anurans respond preferentially to the calls of their own species. In two circumstances reported by A. P. Blair (1942, 1947b), males have been observed to proceed away from the calls of their own species toward those of another species. W. F. Blair (1955a,b, 1956a,b, 1957a,b) has engaged in a series of studies to evaluate the possible significance of differences in mating calls among anurans.

Mating is random in the sense that the male is not selective in his choice of a mate. The literature on the mechanisms of sex recognition in species of Rana and Bufo has been reviewed by Noble and Aronson (1942) and Aronson (1943, 1944). There is typically no preamplexus recognition; a sexually excited male grasps any individual within reach. Sex recognition thus constitutes the discrimination of a male from a female after the embrace is attempted. In Rana species, the major recognition factor is the relative girth or firmness of the body of the sexes; the male maintains his clasp on individuals having a large girth. The warning croak of an embraced male also facilitates recognition. In Bufo species, the mechanism depends primarily on the warning vibrations of a misclasped male; females do not emit these vibrations. Recently, Martof (1953a) presented data indicating a primitive type of territorial behavior in the green frog, *Rana clamitans*. Adult males in the breeding ponds were observed to be spaced at uniform distances of approximately 2–3 meters. Repetitious trial and error testing among the males is greatly reduced, thus effectively conserving the energies of the males for reproduction. The maintenance of spatial relations also increases the chance of a female being contacted by a male.

Several investigators (Wright, 1932; Force, 1933; Hamilton, 1934; Ting and Boring, 1939, among others) found that certain anurans require at least 2–3 years of growth before attaining sexual maturity. It is evident, however, that these findings cannot be generalized, as has been the tendency, particularly for the smaller-sized species or those inhabiting regions permitting growth throughout the year. W. F. Blair (1953) demonstrated that the Gulf Coast toad, *Bufo valliceps* can attain sexual maturity at 10 months of age. Jameson (1955) reported that the cliff frog, *Syrrhophus*

marnocki, is sexually mature in three to four months, and that frogs hatched early in the spring can contribute to the fall breeding population. The narrow-mouthed toad can reach breeding maturity twelve months after metamorphosis (Fitch, 1956b). The ability to complete the life cycle in a single year is an important survival factor, particularly in light of the small percentage of juveniles that survive to sexual maturity (W. F. Blair, 1953; Jameson, 1956).

The effects of periodic fluctuations in population size have not been assessed. In anuran species that hibernate, a large number of individuals undoubtedly perish annually during the winter. (In certain regions, the hot, dry summer may be the most hazardous season.) The winter population may become extremely homogeneous. Selection pressures probably decline as the surviving animals emerge under suitable weather conditions to feed and breed. As the population density rises, the active competition of individuals for means of subsistence and mates increases. The activity of predators and parasites is also intensified. It may be reasoned that the adults that survive both periods of struggle are those containing the most favorable genotypes. Survival may be, however, to a certain degree, a matter of chance. It would be of interest to compare the genetic composition of a population from one breeding season to the next. Jameson's data (1956, 1957) on the Pacific tree frog indicate a rapid population turnover in a one-year period. It would seem also that the greatest variability is to be found in the tadpole population. That this is so is reflected in the numerous structural aberrations encountered in large samples of tadpoles (see study by Nichols, 1937).

INTERSPECIFIC HYBRIDIZATION

Concluding remarks will center on the possible effects of interspecific hybridization in modifying or altering a species population. Interspecific gene exchange can occur in nature when members of two sympatric species utilize the same pool simultaneously for breeding purposes. In most cases, the breakdown of preferential mating behavior, if it occurs, results in a wastage of gametes.

Thornton (1955) and Volpe (1956b) found that hybrids between *Bufo valliceps* (Gulf Coast toad) and *B. woodhousei* (Woodhouse's toad) or *B. fowleri* (Fowler's toad) are inviable or sterile. F_1 hybrids from the experimental cross, *B. valliceps* ♀ × *B. woodhousei* ♂ (or *B. fowleri* ♂) die in an early stage of embryonic development. The majority of the reciprocal hybrids are viable, but all are sterile males. A relatively high percentage of natural hybrids may be found in localities where the range of the Gulf Coast toad overlaps those of Woodhouse's toad (in Texas) and Fowler's toad (in Louisiana). W. F. Blair (1956c) found no evidence that the F_1 hybrids in a Texas population are at a selective disadvantage to either parental species. Volpe (unpublished) has observed mismated pairs of *B.*

valliceps and *B. fowleri* in each season for a three-year period in a local population in New Orleans. Moreover, most of the adult F_1 hybrids marked in any one season have been recovered at least one season following, and some have been recaptured two and three seasons following. Unmarked F_1 hybrids have been collected each season, either constituting new arrivals or individuals that escaped detection and marking in the previous season. Either the F_1 hybrids have some selective advantage or a few derived from each year's mismatings survive by sheer chance. It seems unlikely, however, that accidental survival can be the instrumental factor over a considerable span of time.

Comparable results have been reported in other anuran species by Dürken (1935, 1938), Kawamura (1943, 1950) and Montalenti (1933, 1938). Only sterile males resulted from crosses between *Rana arvalis* ♀ and *R. fusca* ♂, as demonstrated by Dürken, and between *R. japonica* ♀ and *R. temporaria ornativentris* ♂, as shown by Kawamura. Montalenti also demonstrated differences in the developmental fate of reciprocal hybrids. Embryos of the cross, *Bufo viridis* ♀ × *B. vulgaris* ♂, are inviable, whereas those of the reciprocal cross can develop beyond metamorphosis. The gonads of the metamorphosed toads, however, did not exhibit any evident abnormalities.

A. P. Blair (1941a) and Gosner (1956) investigated the possibility of gene exchange between two sympatric species of tree frogs, *Hyla crucifer* and *Pseudacris nigrita*. The low viability of the experimental hybrids led both investigators to conclude that occasional hybridization, if it occurs, would have little effect on the population characteristics of the two species.

One of the few situations in which reciprocal hybrids are viable, fertile, and appear to maintain themselves in considerable numbers, is found among the North American complex of toads, *Bufo americanus, B. terrestris, B. fowleri, B. woodhousei*. In localities where two or more of these species coexist, hybrid individuals have been found. No isolating mechanism thus far detected can be regarded as sufficiently strong to permit a complete separation between any two of the species of toads. Habitat and seasonal isolation tend to maintain the integrity of each species, but not in a manner rigid enough to preclude the possibility of hybridization.

A. P. Blair (1941b, 1942, see also summary of Blair's unpublished data in Moore, 1955) provided the first experimental evidence for hybridization between *B. americanus* and *B. fowleri* by demonstrating that laboratory crosses between members of the foregoing species gave rise to viable hybrids. These laboratory hybrids resembled intermediate individuals collected in the field, and the inference was reasonably made that the intermediate forms found in nature owed their existence to interspecific crosses. F_1 males from the experimental cross, *B. americanus* ♀ × *B. fowleri* ♂, were backcrossed successfully to *B. americanus*.

In a population in Bloomington, Indiana, A. P. Blair (1941b) found that an early-breeding *americanus* segment and a late-breeding *fowleri* unit were

connected by breeding aggregations consisting of *americanus*-like, *fowleri*-like, and individuals morphologically diagnosed as hybrids. The hybrid population in St. Joseph County, Michigan studied by Cory and Manion (1955) also showed a progression from early-season predominantly *americanus*-like to late-season predominantly *fowleri*-like individuals. In the Westwood, New Jersey population analysed by Volpe (1952), the data suggest that many of the *americanus* characteristics have been absorbed by extensive hybrid backcrossing to the more abundant *fowleri* individuals. The evolutionary significance of continual backcrossing to one parental species (introgression) is speculative. One outcome would be the imparting of greater variability to the *fowleri* stock. In a sense, such a result is negative for evolutionary progress since the adaptive fitness of the specialized *fowleri* stock would be decreased. Yet the resultant increase of potential variability through introgression would tend to "buffer" the species against changing environments. It is possible that some of the recombination products might be better adapted to the particular environment than the parental species; introgressive hybridization might then result in the emergence of superior genotypes.

The prevailing view is that the two species of toads had been selected in the past during a period of geographical separation for different ecological situations which have since coalesced in many areas in the broad overlap zone. A. P. Blair (1941b, 1942) believes that natural hybridization of *B. americanus* and *B. fowleri* may be of recent origin, brought about by man's activities, such as the clearing of woodland and the damming of streams. There is no evidence, however, that physiological barriers to intercrossing have developed in response to the challenge of hybridization in sympatric areas. On the contrary, there is some indication that certain allopatric populations of the two species have developed characters associated with weak isolating effects (Volpe, 1955b).

A somewhat similar case involves two species (or subspecies depending upon the interpretation) of narrow-mouthed toads, *Microhyla carolinensis* and *M. olivacea*. Intergradation occurs in a narrow zone in eastern Texas and Oklahoma (Hecht and Matalas, 1946; W. F. Blair, 1955b,c). The greatest differences in body size and mating call between the species were found in the overlap zone (W. F. Blair, *op. cit.*). W. F. Blair surmises that these differences may serve to more effectively isolate the two groups in the region of intergradation, and further contends that the data support Dobzhansky's thesis (1940) that isolating mechanisms are reinforced by natural selection in response to the loss of fitness resulting from intercrossing. The efficacy of differences in body size and mating call as isolating mechanisms has yet to be demonstrated. What appears unlikely is the supposition that the differences were selected as a result of the formation of disharmonious hybrid combinations. Critical evidence is lacking that the hybrids are less viable, less fertile, or less well adapted than the parental types. Moreover, the frequency of hybridization may be so low as to preclude the operation of selection.

W. F. Blair (*op. cit.*) essentially demonstrated the presence of geographical gradients in body size and mating call in each species. Specifically, *M. olivacea* shows a west to east decrease in body length, whereas *M. carolinensis* exhibits an east to west increase. The character gradients are such that the smallest individuals of *M. olivacea* and the largest individuals of *M. carolinensis,* on the average, occur in the overlap zone. Now the tenet that isolating mechanisms are reinforced by selection in response to hybridization in sympatric areas implies, as Dobzhansky recognized, that the genes that diminish the frequency or prevent the occurrence of hybridization have positive selective value only in the area of intergradation. Outside the overlap zone the genes would be expected to be neutral, or even deleterious, since they are selected only to enhance isolation. Is it to be assumed then that the "isolating" genes, presumably expressing themselves in body size and mating call, diffuse unopposed by selection into the allopatric areas where there is no risk of hybridization?

A simpler and more plausible interpretation is that the clines demonstrated by W. F. Blair are due to local differences in selection in the range of each species, possibly correlated with environmental gradients. Selection pressures probably vary in intensity among the local populations, and may be severe in the zone of intergradation. The region of overlap, as pointed out by W. F. Blair, is for the most part a disturbed terrain. The two species, differing in their habitat requirements, most likely compete with each other for suitable ecological niches. If differences in body size and mating call, by themselves or associated with other characters, tend to reduce competition between the two species, then selection acting on the total population may augment the differences. The initial character gradient thus becomes steepened in the overlap zone. It is to be noted that the differences in characters become more pronounced as a result of selection reducing competition rather than as a consequence of the production of hybrids, whether ill-adapted or not.

In many studies on evolution, there is a tendency for investigators, of which the writer claims to be no exception, to interpret, or rather "fit", their data to prevailing evolutionary theories, rather than to design experiments to test critically the validity of the current postulates or to exploit new possibilities.

SUMMARY

Studies on polymorphism, quantitative characters, adaptive characters, population dynamics, and interspecific hybridization in frogs and toads have been reviewed and discussed. The fragmentary information on these aspects serves to emphasize the need for detailed, long range investigations on the genetic composition and structure of local populations (demes) of anuran species.

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THE RELATION BETWEEN YOLK UTILIZATION AND DIFFERENTIATION IN THE FROG EMBRYO

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INTRODUCTION

The premise that yolk utilization in the frog embryo is correllated with differentiation is based upon the fact that yolk is converted to cytoplasm and that it is the cytoplasm, to a large measure, which characterizes a cell as differentiated. The importance of the yolk supply in the formation of cytoplasmic proteins, and presumably therefore for differentiation, is an obvious one since this is the evident source of the materials for synthesis of cytoplasmic proteins. In the frog egg about 70 percent of the total protein (Lawrence, Needham, Miall, and Shen, 1944) and 30 percent of the ribonucleic acid (Grant, 1953) is localized in the yolk. Bragg (1939) has indicated that yolk utilization first begins in differentiating cells and there is some circumstantial evidence that certain differentiating tissues are good inductors; for example, the ear or nose vesicle can elicit an extra limb midway between the fore and hind limb buds (Balinsky, 1927), and Niu (1956) has recently shown the inductive action of differentiating endoderm cells.

The yolk platelets are known to be important in determining the polarity of the egg since Ancel and Vitemburger (1948) and Pasteels (1951) have shown that when the fertilized egg is temporarily inverted 135°, and the yolk platelets are dislocated under the influence of gravity, then invagination tends to occur where the greater amount of yolk is in contact with the pigmented cortex. The relocation of yolk can thus alter the prospective dorsal-ventral axis of the embryo. Waddington (1956) believes that the normal formation of the gray crescent seems to carry yolk towards the animal pole. The movement of yolk up through the pigmented cortex to form the gray crescent takes on additional significance since the pigment granules are known to have phosphoprotein phosphatase activity (Barth and Barth, 1954), and this enzyme has been implicated in the utilization of yolk platelets by both Barth and Barth and Flickinger (1956).

The induction of neural tissue from undetermined urodele gastrula ectoderm by solutions of pH 4.0 and 9.7 (Holtfreter, 1947) and the induction of dorsal mesodermal structures (notochord, muscle, pronephros) from ventral mesoderm of the gastrula by an alkaline pH (Yamada, 1950) are particularly interesting in that these pH levels also cause the solubilization of protein from the yolk platelets (Holtfreter, 1946).

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Flickinger (1949) has inhibited yolk platelet utilization in tissue cultures of pro-pigment cells by adding amino acids to the medium. These amino acids are presumably active by virtue of their ability to chelate calcium and magnesium which are apparently necessary for the solubilization of yolk. These cells became highly stellate but did not form pigment. The assumption is that if certain materials in the yolk are not made available to the cell in soluble form, differentiation, in this case melanin formation, does not occur.

MECHANISM OF YOLK UTILIZATION

Although yolk platelets may be solubilized by strong salt concentrations or extreme pH levels, it appears that the enzyme phosphoprotein phosphatase (Harris, 1946, Panijel, 1950, Mezger-Freed, 1953, Nass, 1956) may mediate this solubilization *in vivo* (Barth and Barth, 1954, Flickinger, 1956).

Incubation of yolk platelets, or vitellin (the yolk phosphoprotein fraction), with pigment granules (which possess phosphoprotein phosphatase activity), at a .01 M $CaCl_2$ concentration, demonstrates that as protein phosphorous is hydrolized from vitellin, protein is solubilized. This correlation holds true through the period of early development and the increasing activity of phosphoprotein phosphatase during development is linked with an increasing amount of protein solubilized from the yolk.

If vitellin (the main constituent of yolk) is defined as the water-insoluble phosphoprotein fraction, and livetin as the water soluble yolk protein fraction, resulting from dialysis of 10 percent NaCl dissolved yolk against distilled water, then phosphoprotein phosphatase is not only hydrolizing protein phosphorous, but also converting vitellin to livetin in terms of defining these fractions according to their solubilities.

Regarding this conversion Barth and Barth (1954) gave electrophoretic evidence in support of the idea that yolk protein exists as a complex of two components. Schjeide, Levi, and Flickinger (1955) have obtained similar electrophoretic patterns, and also ultra-centrifuge patterns, for the vitellin fraction which show that it is made up of two main components.

Barth and Barth (1954) believe that phosphoprotein phosphatase action hydrolizes phosphate from the smaller component of the vitellin "complex" and then releases the large component of the complex in a water soluble state. They partially separated the two components by adjusting the pH and then demonstrated that the smaller fraction is a better phosphoprotein phosphatase substrate; this suggests that it is the phosphoprotein.

Flickinger and Schjeide (in press) have separated some of the lighter ultra-centrifugal component, by utilizing a separation cell in the ultra-centrifuge method (Schjeide and Dickenson, 1953) and phosphorous/nitrogen ratios demonstrated that the lighter component is the phosphoprotein as compared to the larger component. The addition of calcium chloride (which is known to solubilize vitellin) to the vitellin complex, and ultra-centrifu-

gal separation of the components, demonstrated that the phosphoprotein component can selectively bind calcium. Livetin preparations did not bind calcium. It would appear that since livetin cannot bind calcium, and since livetin has a much lower P/N ratio than vitellin (Flickinger, 1956), livetin does not contain this small phosphoprotein component. This is also demonstrated by the ultra-centrifuge patterns (Schjeide, Levi, Flickinger, 1955), where the lighter component is absent; however, the larger component is seen in both vitellin and livetin preparations although livetin has several components not present in vitellin. Also the large component is identical in the two fractions as indicated by electrophoresis of mixed samples of vitellin and livetin (dissolved in an alkaline buffer) where only one large component was seen, thus suggesting that the two large components had melded together and were similar. Agar plate serology also indicates partial similarity of vitellin and livetin presumably due to their identical large components (Flickinger and Rounds, 1956). If the anti-livetin serum is absorbed with adult frog serum, and then reacted against centrifugal supernate fractions from the oocyte and larval stages, the extra line in the latter case indicates the release of a livetin-like protein into the cytoplasm by the larval stage.

The fact that calcium can bind protein phosphorous groups indicates that these phosphate groups play a role in holding the vitellin complex together, probably by electrostatic or hydrogen bonding of the phosphate groups to groups of the larger component. This type of bond is ruptured by the same agents which solubilize vitellin (strong salt concentrations and low and high pH solutions). The removal of some of these phosphate groups by phosphoprotein phosphatase activity would also tend to weaken the linkage between the small phosphoprotein and the larger component and release the latter in its water-soluble state. Flickinger and Schjeide (in press) have shown that isolated phosphoprotein can bind larval livetin and be precipitated and therefore the complex is reversibly dissociable experimentally.

That the hydrolysis of phosphoprotein phosphate and subsequent solubilization of protein occurs during development seems apparent from several lines of evidence. First; the phosphoprotein phosphatase activity increases up through development; second, the ratio of livetin (water-soluble yolk protein fraction) to vitellin (water-insoluble yolk protein fraction) increases during development; and third, agar plate serology demonstrates that a livetin-like component has been released from the yolk into the soluble cytoplasm between the egg and larval stages.

TEMPORAL AND REGIONAL PATTERN OF YOLK UTILIZATION

The temporal sequence of yolk utilization and its regional distribution seem to be important in accounting for the animal-vegetal and dorsal-ventral respiratory gradients (Barth, 1942, Flickinger and Blount, in press) and also the regional differences of incorporation of $C^{14}O_2$ (Flickinger, 1954).

It seems likely that these metabolic gradients are due to a regional distribution of yolk, and the utilization of this yolk at different times during development. The measurement of the respiration of embryonic cells in tissue culture, using the Cartesian diver technique (Flickinger, 1949), has shown an increase in oxygen consumption that follows the conversion of yolk to cytoplasm and provides a direct correlation between yolk utilization and respiration.

Rounds and Flickinger (unpublished data) have investigated the problem of yolk utilization in relation to induction by cutting out and separating the inducing chorda mesoderm and reacting gastrula ectoderm at various stages during gastrulation and neurulation and performing microchemical assays upon centrifugal fractions of homogenates of these parts of the embryos. One product of this investigation has been the discovery that increased solubilization of yolk protein and ribonucleoprotein takes place in the chorda before such an increase occurs in the overlying ectoderm. Naturally this brings up the possibility that the inducing material that diffuses from the chorda to the overlying ectoderm might possibly be a product of the solubilization of the yolk platelets. They noted that solubilized yolk ribonucleic acid in the chorda localizes on the granular fraction (which includes the microsomes); and a temporary decrease in the quantity of granular RNA in the chorda was seen at the termination of gastrulation. However an increase in granular RNA does not occur in the ectoderm until the early neurula stage.

In another of their experiments radioactive tissues (dorsal lip mesoderm, ventral lip mesoderm and gastrula ectoderm) labeled with $C^{14}O_2$ have been grafted against non-radioactive tissues (gastrula ectoderm and dorsal lip mesoderm) and after 8 hours of culture the tissues were separated, homogenized, and the proteins and nucleic acids of the originally non-radioactive tissues were precipitated with trichloracetic acid. The acid insoluble fractions were washed several times and after combustion CO_2 was collected as $BaCO_3$ and counted. In some cases the nucleic acids were extracted from the acid insoluble fraction and counted. The activities of the acid soluble fractions of the radioactive tissues were also counted. The results were expressed as the specific activity of the acid insoluble, or nucleic acid fraction of the originally non-radioactive tissue, based upon a standard level of acid-soluble activity for all the radioactive tissues. The highest activity was obtained in the case of non-radioactive gastrula ectoderm cultured with radioactive dorsal lip mesoderm, where induction occurred, indicating a greater passage of radioactive compounds that come to reside in the acid insoluble fraction of the originally non-radioactive tissue. Since the greater level of activity was found in the nucleic acid fraction, this suggests the diffusion of a nucleoprotein during induction.

However, these results may be explained in two ways. Either low molecular-weight compounds are diffusing from the chorda to the ectoderm, and then are incorporated into the protein-nucleic acid fraction, or proteins (nu-

cleoproteins) move between tissues at the time of induction. The former possibility seems unlikely since membranes, that would allow small molecules to pass, block induction (Brachet, 1950) and the latter alternative is favored since Niu and Twitty (1953) have demonstrated that non-dializable nucleoproteins collected from cultures can cause induction. Since the levels of acid insoluble or nucleic acid activity were always based upon a similar level of acid soluble activity in the radioactive tissue, the greater activity of the acid-insoluble fraction in the induced ectoderm is probably not due to a larger pool of small molecular weight radioactive molecules in the radioactive tissues, and the assumption is that some large molecular weight substances (nucleoproteins) pass from the inducing to the reacting tissue. After that passage the synthesis of the proteins of the neural tissue undoubtedly is partially dependent upon the pool of acid soluble radioactive compounds.

The possibility of protein or nucleoprotein transfer between the chorda and the overlying ectoderm was also tested in the following manner by Rounds and Flickinger (unpublished data). An antiserum to gastrula stages of *Rana pipiens* was obtained which had a homologous titer of 1/512 (anti-serum dilution) against a 0.1% protein extract of the homologous antigen. This anti-serum was tested against 0.1% protein extracts of *Taricha torosa* (*Triturus torosus*) gastrulae and neurulae and the cross-reaction titer was 1/8 (anti-serum dilution) in either case. Then a number of explants of Rana dorsal lip, fused with Taricha gastrula ectoderm, were made. These fused tissues were explanted for 3-4 days and then Taricha tissues were carefully dissected away from the Rana tissues. The Taricha tissues, thus obtained, were pooled, homogenized and centrifuged. The supernates were adjusted to a 0.1% protein concentration and reacted against the antiserum in precipitin tubes in order to detect whether any Rana protein (nucleoprotein) from the dorsal lip material had passed into the Taricha ectoderm. The cross reaction titer of Taricha ectoderm with Rana antiserum was found to be 1/32 (anti-serum dilution) as opposed to a 1/8 antiserum dilution titre of the control Taricha tissue which had not been in contact with Rana Dorsal lips. This suggests that Rana proteins had moved over into the cells of the Taricha tissue.

NEURAL INDUCTION DUE TO CO_2 SHOCKS

If a regional and temporal sequence of yolk utilization, which results in a regional and temporal sequence of synthesis of cytoplasmic protein in the embryo, is indeed a causal factor in amphibian morphogenesis, then the most critical data which might support this hypothesis are to be derived from biological experiments.

Thus it was hoped, by culturing bits of ventral mesoderm and ectoderm from *Rana pipiens* gastrulae under conditions which might alter the nutrient supply and rate of metabolism, to change the normal pattern of protein synthesis in the tissues and perhaps to modify their pathway of differentiation.

Before any attempts were made to elicit the formation of dorsal structures in isolated ventral halves of frog early gastrulae by experimental procedures, a number of ventral halves were isolated and cultured to determine if dorsal structures could spontaneously differentiate as was found to be the case by Dalcq and Huang (1948) for ventral halves from Triton gastrulae. No nervous, chordal, nephric, or muscular tissues were obtained in such "control cultures" of ventral halves of *Rana pipiens.*

In the experimental cases ventral explants were cultured at higher temperatures (28–33° C.), and in various nutrient media. The explants were also subjected to brief shocks with alkaline and acid media (Flickinger, unpublished data).

Of all these treatments the use of CO_2 shocks provided the only positive results. Neuroid tissue and brain structures were frequently noted in explants that had received CO_2 shocks of a ten minute to one hour duration (pH 3.7). The interpretation of the mechanism of CO_2 induction is that the acid shock released soluble protein from the yolk platelets thus initiating differentiation. Apparently pH induction can be obtained with the frog embryo as well as the urodele embryo.

SUMMARY

The data reviewed suggest that the utilization of yolk platelets to form cytoplasmic proteins is associated with embryonic differentiation in the frog embryo. The mechanism of yolk utilization has been linked to the activity of the enzyme phosphoprotein phosphatase. To speculate from the data, the temporal and regional patterns of yolk utilization, which account for the temporal and spatial patterns of synthesis of cytoplasmic proteins, might possibly allow a preferential hierarchy of cell and tissue specializations to proceed. At the present time it is difficult to imagine the basis of this possible sequence or hierarchy of cellular differentiation. Apparently the dorsal structures (chorda, muscle) form where cytoplasmic protein is first formed. Recently much emphasis has been placed upon the influence of the extra-cellular environment in relation to cellular differentiation. However, since acid and alkaline shocks can elicit the differentiation of dorsal tissues from ventral regions (without the presence of any inductor tissues), this implies that the materials that will make up the environment are potentially present throughout the egg. The prior solubilization of protein (including ribonucleoprotein) in the chorda, as opposed to the ectoderm, places a good deal of emphasis upon the time factor in differentiation. This suggests the speculation that the organizer area (chorda and somites) forms where the first and most active formation of cytoplasmic protein occurs.

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ANTHOCYANIN SYNTHESIS IN MAIZE—A GENE SEQUENCE CONSTRUCTION[1]

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As pointed out by Rhoades (1952), a study of anthocyanin synthesis in maize promises to be rewarding. The several anthocyanin-affecting factors available, and their variety of expressions, provide attractive material for analysis. A necessary preliminary to studies of gene action in this synthesis is the establishment of a tentative gene sequence, for which unique visible and chemically determined phenotypic expressions of the individual pigment-controlling factors are needed. This report deals with such an expression of the aleurone-color intensifier factor and a sequence which can be constructed from data on its interaction with other color factors.

The inheritance of anthocyanin pigmentation in the aleurone tissue of maize has been summarized by Emerson et al. (1935). In order for color to appear in this tissue, factors A_1, A_2, *Bz*, *C*, and *R* must be present in the condition noted. If any of these is in the recessive condition, or if the dominant allele C^i is present, the aleurone is colorless, except in the case of *bz*, which is associated with a bronze-colored aleurone having a small amount of anthocyanin (Rhoades, 1952). If *Pr* is present, the pigment in the aleurone layer is purple; if *pr* is present, the pigment is red. The recessive intensifier, *in*, results in a greatly increased quantity of pigment in the aleurone layer, to such an extent that intense kernels of both *Pr* and *pr* constitution appear black.

Chemical and genetic studies relating to the establishment of a sequence include some of the very earliest attempts to relate the gene to its products. East and Hayes (1911) reported a study of the effects of the *Pr-pr* factor pair, and suggested that the two pigment types might differ in level of oxidation of the same color base. This has been confirmed by McClary (1942) and Coe (1950), who found that the anthocyanidin of *Pr* aleurone is cyanidin, while that of *pr* is pelargonidin. The classical work of Sando et al (1935), demonstrating the accumulation of parallel molecular species, differing only in level of oxidation, has provided a useful unique expression of A_1 action, even though the early interpretation of this action is now in doubt (Laughnan 1946, 1950). On the basis of visual observa-

[1]Contribution from Crops Research Division, ARS, USDA, and Department of Field Crops, University of Missouri. Missouri Agricultural Experiment Station Journal Series No. 1791.

tions by Laughnan (1951), chemical studies utilizing the established properties of a_1 plant tissues have supported his suggestion that A_1 precedes A_2 and *Bz* in action, and have indicated that *Bz* precedes A_2 (Coe, 1954), although this latter order is by no means clearly established. The accumulation of leucoanthocyanin in the aleurone tissue of colorless a_2 kernels and the relation of *Pr* to this accumulation has led to the suggestion that the action of *Pr* precedes that of A_2 (Coe, 1955).

THE PERICARP SHEEN OF INTENSE KERNELS

In the course of production of various combinations of aleurone color factors to be used in projected chemical studies, it was found that ears having purple kernels with *in* segregating show a segregation for a bronze-metallic sheen in the pericarp. This sheen is perfectly associated with *in*. The pericarp is a maternal tissue overlying the endosperm, and the sheen can thus be considered as an example of metaxenia, although since *in* is treated as a recessive it would be more appropriate to consider *In* as showing metaxenia in the elimination of sheen from the pericarp. Very likely the comment, "—sometimes with a brassy appearance," used by Fraser (1924) in the original description of *in* refers to this sheen. The color-intensification effects of *in* are so much more clear on a *pr* background that *in* has been maintained and utilized almost entirely in red-aleurone stocks, where the sheen effect is only rarely observed.

When the acid test for anthocyanin is applied to separated bits of sheen-bearing pericarp, there is no change in color. The pigment is thus not anthocyanin, but could easily be a flavonoid derivative, perhaps similar to the brown pigments accumulated in the plant tissues of certain genetic types (see Laughnan, 1950). Presumably the sheen-like pigment arises through the conversion, in the pericarp, of diffused anthocyanin relatives from the aleurone tissue.

DIFFERENTIAL CONTROL OF SHEEN PRODUCTION IN COMBINATIONS

Preliminary observations on combinations involving other aleurone factors were easily obtained in F_2 of such crosses as a_1 *In* by A_1 *in*, where one-sixteenth of the kernels are observable for sheen on the colorless kernels. These observations have been confirmed by second-generation tests to check for *in*; the results are listed in table 1. The color in the pericarp varies with the constitution of the underlying kernels. Purple kernels have a bright gold-metallic pericarp, a_1 kernels bright yellow-brown, a_2 kernels dull buff, and *bz* kernels brown with reddish tinge. These color types are exactly parallel to those observed in husk and sheath tissues of mature plants of *In In* constitution (Coe, 1954). In fact, the presence of a small amount of anthocyanin pigment in the aleurone and plant tissues of *bz* individuals (Rhoades, 1952) is reflected also in the pericarp of *bz in* kernels, where a test with dilute hydrochloric acid shows a small amount of anthocyanin to be present.

TABLE 1

APPEARANCE OF SHEEN IN COMBINATIONS OF *in* WITH VARIOUS OTHER ALEURONE FACTORS. CONSTITUTION IS A_1 A_2 *Bz C R in in* UNLESS OTHERWISE NOTED

Constitution	Pericarp Sheen
Pr	present
pr	occasional
a_1 *Pr*	present
a_1 *pr*	occasional
a_2 *Pr*	present
bz Pr	present
c Pr	absent
C^1 *Pr*	absent
r Pr	absent

An elegant demonstration of the effects listed in table 1 has been found by Dr. M. G. Nuffer, on an ear produced for other purposes. In the cross a_1 a_1 *in in R r* Dt_3- × a_1 a_1 *in in R r* Dt_3-, three-fourths of the kernels have brown pericarp and dotted aleurone (effect of Dt_3 on *a*), and one-fourth have colorless aleurone, colorless pericarp, (the *r r* kernels). Further, a portion of the dotted kernels show a mottled brown appearance, comparable to the mottling seen in *R r r* colored kernels. In contrast to the brown pigment in all other types of kernels, including sib kernels on the same ear, this brown mottling is stronger in the aleurone tissue than in the pericarp; the explanation for this difference is not clear.

A GENE SEQUENCE CONSTRUCTION

Since *in* shows a dosage effect (Fraser, 1924), and no deficiency tests have been applied, it is impossible to make definite conclusions as to the action or inaction of *in*; however, its precise action, whether through an inhibition of anthocyanin synthesis by the "dominant" allele, or through an enhancement by the "recessive", does not affect considerations of sequence. Since a_1 a_1 kernels show the pericarp effect of *in*, and A_1 is thus unnecessary for synthesis of the pigment, it is assumed that the effects of *in* are expressed before those of A_1 in a presumed simple linear sequence for anthocyanin synthesis. Similarly, *in* precedes A_2 and *Bz*. Preliminary experiments on a_2 *in* aleurone tissue, where the quantity of leucoanthocyanin (see Coe, 1955) is found to be distinctly increased over a_2 *In*, are in agreement with the *In*-A_2 order. Since *C* and *R* are necessary for the expression of *in*, it can be assumed that these two factors precede *in*. It is interesting and perhaps significant that the two groups so separated are natural categories. *C* and *R* show their effects only in the aleurone tissue, not in the husks and sheaths; A_1, A_2, and *Bz* are active in both aleurone and plant tissues. Concurring observations of the pericarp effects have been made on both segregating and homozygous ears, so it is apparent that the genetic constitution of the pericarp itself does not affect *in* expression. Consequently, it would appear that the precursors of action of A_1, A_2,

and *Bz* are all capable of diffusing into the pericarp, perhaps even in purple intense kernels. The diffusibility of the product of *C* action, discussed by Rhoades (1952), lends support to the suggested gene sequence, and suggests that the majority of precursors of anthocyanin are diffusible. It should be noted that purple intense kernels frequently have a gradient of pigment extending into the inner endosperm, even though the pericarp of such kernels contains no pigment; this suggests a diffusion of precursors, but not of anthocyanin itself.

Combining these conclusions with the previously discussed data indicating that A_1 precedes *Bz* and A_2, that *Bz* may precede A_2, and that *Pr* precedes A_2, the simplest interpretation is as follows:

$$\underset{(\text{- - } Pr \text{ - - - - - - - - - - - - - - - - - -})}{\overset{(C,\ R)}{\longrightarrow}\ \overset{In}{\longrightarrow}\ \overset{A_1}{\longrightarrow}\ \overset{Bz}{\longrightarrow}\ \overset{A_2}{\longrightarrow}} \text{ anthocyanin}$$

Repeated attempts to find distinctive effects of *C* and *R* by paper chromatography have been unsuccessful.

It is considered unlikely that further static studies of mature tissues will give definitive confirmation or will result in significant changes in the suggested sequence. Since shunt pathways of the sort suggested by Laughnan (1951) can exist, not only in outwardly confusing systems, but also in apparently simple systems, it must be concluded that clear establishment of a gene sequence, in any organism, rests upon a study of the stage of active synthesis. Lacking such a study it is possible, through application of appropriate theoretical conditions, to construct a sequence in any order. For example, *In* might be considered to act after A_1 or a_1 by affecting not only the product of A_1 (leading to anthocyanin), but also its precursor (leading to brown pigments); or *In* might act before *C*, with the precursor of *C* action being non-diffusible, or incapable of conversion to brown pigment in the pericarp.

SUMMARY

Through a unique expression of the aleurone intensifier factor in maize and its relationship to other major factors controlling anthocyanin production, a sequence of gene action has been constructed, based upon earlier studies relating to this sequence. The proposed sequence is (*C, R*); *In;* A_1; *Bz*; A_2, with the effects of *Pr* expressed sometime before those of A_2. This sequence is based upon the simplest assumptions and is subject to drastic revision. It is suggested that confirmation of such sequences requires a study of the active synthetic stages.

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INTER-POPULATION HYBRIDS IN OPEN-POLLINATED VARIETIES OF MAIZE[1]

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Heterosis in the F_1 of crosses of geographically different populations and F_2 breakdown to a level of performance equal or inferior to the parents has recently been reported in Drosophila. Vetukhiv (1954) used viability as a measure of fitness in intra-specific geographic strain crosses in three species of Drosophila, *D. pseudoobscura, D. willistoni, and D. paulistorum.* The results for inter-strain crosses within *D. pseudoobscura* and *D. willistoni* showed F_1 hybrids superior in viability to parent stocks with F_2 mean performance equal or inferior to the average of the parents. Brncic (1954) found similar results in *D. pseudoobscura* strain crosses. Wallace (1955) studied developmental rate in *D. melanogaster* strain crosses from collections representing a wide range of geographic localities. The nature of the results with this cosmopolitan species was of the same type as reported by Vetukhiv and Brncic, namely, F_1 heterosis and performance of advanced generations below the parental types. F_1 and parent lines of *D. melanogaster* were found by King (1955) to show equal resistance to DDT but lower resistance resulted in the F_2 generation. The parent populations, developed from sampling a single wild stock, were separately subjected to over twenty generations of artificial selection to DDT resistance at which time the resistance developed by each was approximately five times that of the control stock. Although the parents were phenotypically indistinguishable, the genetic systems developed in each were different and ill-adapted to each other as evidenced from the lower F_2 performance.

The evidence in maize from populations derived from crossing inbred lines has not favored the existence of appreciable amounts of epistasis in the action of genes concerned with yield of grain and other plant characteristics. Neal (1935), Stringfield (1950) and Sentz *et al.* (1954) all reported F_2 performance in such populations approximately mid-way between F_1 and parental lines.

Performance at varying levels of heterozygosis in material developed from highly selected inbred lines of maize does not provide a satisfactory

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basis for comparison with findings in strain crosses of Drosophila where the F_2 generation has been found equal or inferior to parents. The extreme inbreeding depression in yield in maize accompanying the fixation of deleterious genes results in low yielding inbred parents when compared with the heterozygous material of the hybrid population. The open-pollinated varieties of maize, which, in many respects, are comparable to geographical strains of Drosophila do provide the basic stock for development of material needed in such a study. Heterosis in crosses of maize varieties was observed by numerous investigators prior to 1920 and more recently by Robinson *et al.* (1956). The purpose of the study reported here was to determine the level of performance of advanced generations of variety-crosses, relative to the F_1 and parent varieties as a source of evidence on the importance of epistasis in such plant populations.

MATERIAL AND RESULTS

The Jarvis, Indian Chief and Weekley varieties were used to prepare the F_1, F_2 and backcross generations of the three possible crosses of these varieties. The parental varieties and crosses were compared in an experiment utilizing a randomized block design with six replications at two locations in 1955. Duplicate plots of the F_1 and F_2 crosses occurred in each replication of the Jarvis-Indian Chief, Jarvis-Weekley and Indian Chief-Weekley populations. The data for the four characters measured are presented in Table 1.

TABLE 1

MEAN PERFORMANCE OF PARENTS, F_1, F_2 AND BACKCROSS GENERATIONS IN THREE POPULATIONS PRODUCED FROM CROSSING OPEN-POLLINATED VARIETIES OF MAIZE

Character	Generation	Populations		
		Jarvis-Indian Chief	Jarvis-Weekley	Weekley-Indian Chief
Yield (lbs/plant)	Varieties	.36	.37	.42
	F_2	.39	.40	.47
	F_1	.40	.44	.50
	Backcross	.38	.42	.44
Ear Length (inches)	Varieties	6.53	6.77	6.90
	F_2	6.67	7.01	6.78
	F_1	6.80	7.15	7.13
	Backcross	6.54	7.13	6.84
Ear Diameter (inches)	Varieties	1.72	1.76	1.73
	F_2	1.76	1.75	1.78
	F_1	1.80	1.81	1.83
	Backcross	1.77	1.76	1.76
Ear Number	Varieties	1.28	1.27	1.43
	F_2	1.30	1.32	1.51
	F_1	1.19	1.33	1.43
	Backcross	1.28	1.31	1.45

The relative performance of the entries within the populations were similar at each of the two locations and therefore only combined results have been presented. The standard errors of the means for each character were approximately the same size in the three populations. These were as follows: yield, .02; ear length, .09; ear diameter, .02; and ear number, .04.

DISCUSSION AND CONCLUSIONS

The character of major interest is grain yield, not only for economic consideration, but from the viewpoint of providing some measure of "fitness" in considering the evolutionary development of the genetic system within the varieties. The result is essentially the same in each population; namely, that the F_2 performance is intermediate between the average of parent varieties and the F_1 of the variety cross. The average of the two backcrosses and F_2 are expected to perform equally in the absence of epistasis. Deviations from this expectation are not considered of sufficient magnitude to be disturbing.

Heterosis in yield in the variety cross is approximately 20 percent in the Jarvis-Weekley and Indian Chief-Weekley populations and 9 percent in Jarvis-Indian Chief. It is in the latter population that F_2 performance gave the largest deviation from intermediacy between parents and F_1. Repeated observations of this comparison by the authors, some of which have been published (Robinson, *et al.* 1956), indicates the F_1 cross of the Jarvis and Indian Chief varieties also excels the parents by approximately 20 percent. It is likely that F_2 performance, relative to parents and F_1 does not differ in any of the populations studied.

Ear length and ear diameter exhibit less heterosis than grain yield and, in general, the F_2 is intermediate between parents and F_1. In two instances the F_2 is slightly inferior to the average value obtained for the parent varieties. The number of ears borne on each plant in the prolific varieties of maize shows a relationship different in heterozygous performance from other characters that have been studied. Sentz *et al.* (1954), in populations developed from crossing inbred lines, found a diminished accumulative effect in the expression of this character with added increments of heterozygosis. The maximum number of ears per plant occurred between the 25 percent and 75 percent levels of heterozygosity in three hybrid populations. In two of the populations studied here the F_2 mean performance in ear number excels the F_1. This suggests the existence of an internal balance in the physiological development of the plant in which the interrelationship of the number of ears and size of ears changes at different levels of heterozygosity. This may be closely associated with changes in morphological development as general plant vigor is increased. The larger number of smaller ears in the F_2 generation, relative to the F_1, does not necessarily mean that, under a more favorable environment, the F_1 generation of material of the type studied here may not produce maximum yield of grain from a larger number, as well as larger size, ears.

Although the evidence reported does not suggest the presence of important epistatic combinations of genes conditioning yield in maize, it does not eliminate epistasis as a factor in such populations. The possibility remains that different kinds of non-allelic gene interactions may exist but are so completely equalized as to lead to the intermediate F_2 observed. The populations used may be similar to the extent that some of the same loci are homozygous in the three varieties and would exhibit epistatic effects if heterozygous in the hybrid. The conclusion drawn from these results is that they offer no evidence for the existence of important amounts of epistasis. Although little direct information is available, the results from genetic variance studies in the parent varieties used (Robinson *et al.* 1955) indicate that of the total genetic variance arising from segregating loci, epistatic variance may contribute a minor portion. Further work is in progress to provide more conclusive information on this question.

It is likely that varieties of maize may be found which will exhibit an effect due to epistasis in the variety crosses. If so, it will probably occur in populations having a more diverse origin than those used in this study. These varieties have been developed in close proximity with regard to geographical localities and for adaptation to similar environmental conditions. Thus, although they have diverged sufficiently so that the cross is heterotic, further genetic diversity might give a breakdown in performance following recombination. It would be of interest if experiments, similar to the one discussed here, were performed using varieties of maize from widely different localities. However, the varieties used should mature at approximately the same time in order to subject all genotypes to the same environmental conditions.

SUMMARY

Crosses between three open-pollinated varieties of maize exhibit heterosis in yield in the F_1 generation and the F_2 performance is intermediate between parents and F_1. These findings are in contrast to those in Drosophila where the F_2 has been found equal or inferior to parent strains in characters relating to fitness and they have been interpreted as evidence for the importance of epistasis. While the evidence does not favor important epistatic effects in the gene action conditioning yield in maize, the inconclusiveness of such results for its detection are recognized. The degree of genetic diversity of the parent varieties of maize with particular reference to the possibility of non-segregating loci in these variety crosses in contrast to populations developed from more divergent parents may account for the difference in results with Drosophila and maize.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

TESTS OF CHEMICAL SUBSTANCES AND SPECIFIC GENES FOR THEIR EFFECTS ON TUMOR INCIDENCE IN *D. MELANOGASTER*

The author has reported previous experiments demonstrating that tumor frequency is intimately related to well known tryptophane metabolism associated with eye-color in Drosophila (Kanehisa 1954, 1955, 1956). The present investigation represents a further inquiry into this relationship.

The author is greatly indebted to Professor D. F. Poulson of Yale University for his keen interest in this work and reading the manuscript of this paper.

The relation between tumor-incidence and some chemicals concerned in tryptophane metabolism. The flies used here were derived from *tu, cn tu, v tu,* and *tu st* strains which had been previously established (Kanehisa 1954, 1956). The larvae from single pair matings of flies from these tumor strains were respectively bred on 5 percent dead yeast media with particular chemicals added in Sörensen's phosphate buffer solution, and the tumor-incidence in the emerging adult flies was determined. As shown in table 1,

TABLE 1

EFFECTS OF XANTHURENIC ACID (0.5 MG PER ML MEDIUM) ON TUMOR-INCIDENCE IN TUMOR STRAINS IN *D. MELANOGASTER*

Tumor strains	*tu*	*cn tu*	*tu st*
Control	43%(56/130)*	74%(67/91)	85%(78/92)
Experiment	77%(78/102)	85%(132/159)	87%(168/193)

( )* shows the number of flies of tumor over the total number of flies.

the effect of xanthurenic acid (0.5 mg per ml of medium) on the tumor-incidence is remarkable. The tumor-incidence of *tu* strain increases strikingly up to the level of the *v tu* or *cn tu* strain, and that of *cn tu* strain increases slightly. There is no significant increase of tumor-incidence in *tu st* strain. The effects of l-hydroxy tryptophane hydrochloride (10^{-2}, 10^{-1}, and 1 M in concentration), of indole (10^{-2} to 10^{-6} M in concentration), and of cystine (0.01, 0.1, 0.5, and 1 g per liter in concentration) were explored. No significant differences in the effects of these chemicals on the tumor-incidence were found (tables 2, 3, and 4). No effects of indole in 10^{-2} to 10^{-6} M in concentration were detected because of the severe toxicity of this chemical.

TABLE 2

EFFECTS OF 1-HYDROXY TRYPTOPHANE HYDROCHLORIDE ON TUMOR INCIDENCE IN TUMOR STRAINS IN *D. MELANOGASTER*

Tumor strains	Control	Experiment		
		10^{-2}M	10^{-1}M	1M
tu	40%(55/138)	40%(39/98)	43%(126/221)	47%(81/173)
tu st	82%(98/120)	87%(233/268)	86%(142/165)	80%(214/268)

TABLE 3

EFFECTS OF INDOLE ON TUMOR INCIDENCE

Tumor strains	Control	Experiment		
		10^{-6}M	10^{-7}M*	10^{-5}–10^{-2}M
tu	40%(55/138)	45%(50/111)	41%(44/107)	Death in eggs
cn tu	75%(74/98)	77%(52/67)	75%(71/94)	and 2nd instar
tu st	82%(98/120)	84%(74/88)	81%(113/140)	larvae

*In 10^{-7}M experiment, the tumor-incidence was calculated in the 3rd instar larvae because of the failure of larva to metamorphose.

TABLE 4

THE EFFECTS OF CYSTINE ON TUMOR INCIDENCE

Tumor strains	Control	Experiment			
		0.01 g/1	0.1g/1	0.5g/1	1g/1
tu	40%(40/101)	46%(45/98)	40%(48/122)	44%(64/145)	47%(50/107)
tu st	79%(103/131)	77%(100/130)	86%(77/90)	83%(117/141)	79%(81/103)

The effects of trans-cinnamic acid and maleic acid hydrazide. Trans-cinnamic acid (0.05, 0.01, 0.005, 0.001, and 0.0001 percent in concentration) and maleic acid hydrazide (0.5, 0.25, 0.1, and 0.005 percent in concentration) were respectively added to 5 percent dead yeast medium. The eggs of the flies from *tu* and *tu st* strains were laid on these media and the tumor-incidence was examined. These results are shown in tables 5 and 6. No effect of maleic acid hydrazide on the tumor-incidence was

TABLE 5

THE EFFECTS OF TRANS-CINNAMIC ACID ON TUMOR INCIDENCE IN *TU* AND *TU ST* STRAINS IN *D. MELANOGASTER*

Tumor strains	Control	Experiment				
		0.0001%	0.001%	0.005%	0.01%	0.05%
tu	44%(55/124)	21%(21/102)	15%(18/121)	41%(40/97)	27%(20/73)	Death
tu st	81%(139/171)	29%(61/210)	37%(52/141)	68%(73/107)	20%(14/74)	in eggs and larva

TABLE 6

THE EFFECTS OF MALEIC ACID HYDRAZIDE ON TUMOR INCIDENCE IN *TU* AND *TU ST* STRAINS IN *D. MELANOGASTER*

Tumor strains	Control	Experiment			
		0.05%	0.1%	0.25%	0.5%
tu	43%(73/170)	46%(41/89)	41%(41/99)	40%(37/93)	41%(51/124)
tu st	84%(113/135)	87%(87/100)	89%(110/124)	81%(69/85)	79%(58/74)

observed in 0.1 and 0.05 percent except for a remarkable retardation of larval development. In 0.5 and 0.25 percent almost all the eggs were dead because of severe toxicity of this chemical. On the other hand, as clearly shown in table 5, at the concentrations of 0.01, 0.001, and 0.0001 percent, trans-cinnamic acid showed a remarkable effect in reduction of tumor-incidence.

Effects of some eye color genes on tumor incidence. The author has carried out some matings between the tumor bearing flies from *bw tu* strain in *D. melanogaster* and a number of well known eye color genes to test the effects on tryptophane metabolism (Kanehisa 1954, 1956). This report relates to some crosses between the flies from *tu* strain derived from the previous matings and those from e^{11}, w^a, w^e, *ca*, and *cm* strains in *D. melanogaster*. After the establishment of new tumor strains with one of these eye color genes, the tumor-incidence in these was examined over a period of 4 to 5 generations. The results are given in table 7. These differ

TABLE 7

SOME COLOR GENES AND TUMOR INCIDENCE

Matings	Strains newly established		tumor	+	total	tumor-incidence (%)
tu × e^{11}	*tu* e^{11}	♀	261	253	514	45(458/1009)
		♂	197	298	495	
tu × w^a	*tu* w^a	♀	308	198	506	65(605/936)
		♂	297	133	430	
tu × w^e	*tu* w^e	♀	111	73	184	64(223/348)
		♂	112	52	164	
tu × *ca*	*tu ca*	♀	39(11)*	30(58)	69	56(54/97) 20(19/97)
		♂	15(8)	13(20)	28	
tu × *cm*	*tu cm*	♀	396	250	646	58(769/1322)
			76	152	228	
		♂	373	303	676	31(138/443)
			62	153	215	

( )* shows the number of flies in which it would not be determined whether the fly has a tumor.

from the previous genetical results, since no significant difference between the tumor-incidence of *tu* strain and of *tu* e^{11} and *tu ca* strains were observed, although in $w^e tu$ and $w^a tu$ strains the tumor-incidence showed a small significant increase, compared with the tumor-incidence of the *tu* strain. In the case of *cm tu* strain two groups of the tumor-incidence were obtained, some pair matings giving the higher values, the others the lower values.

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